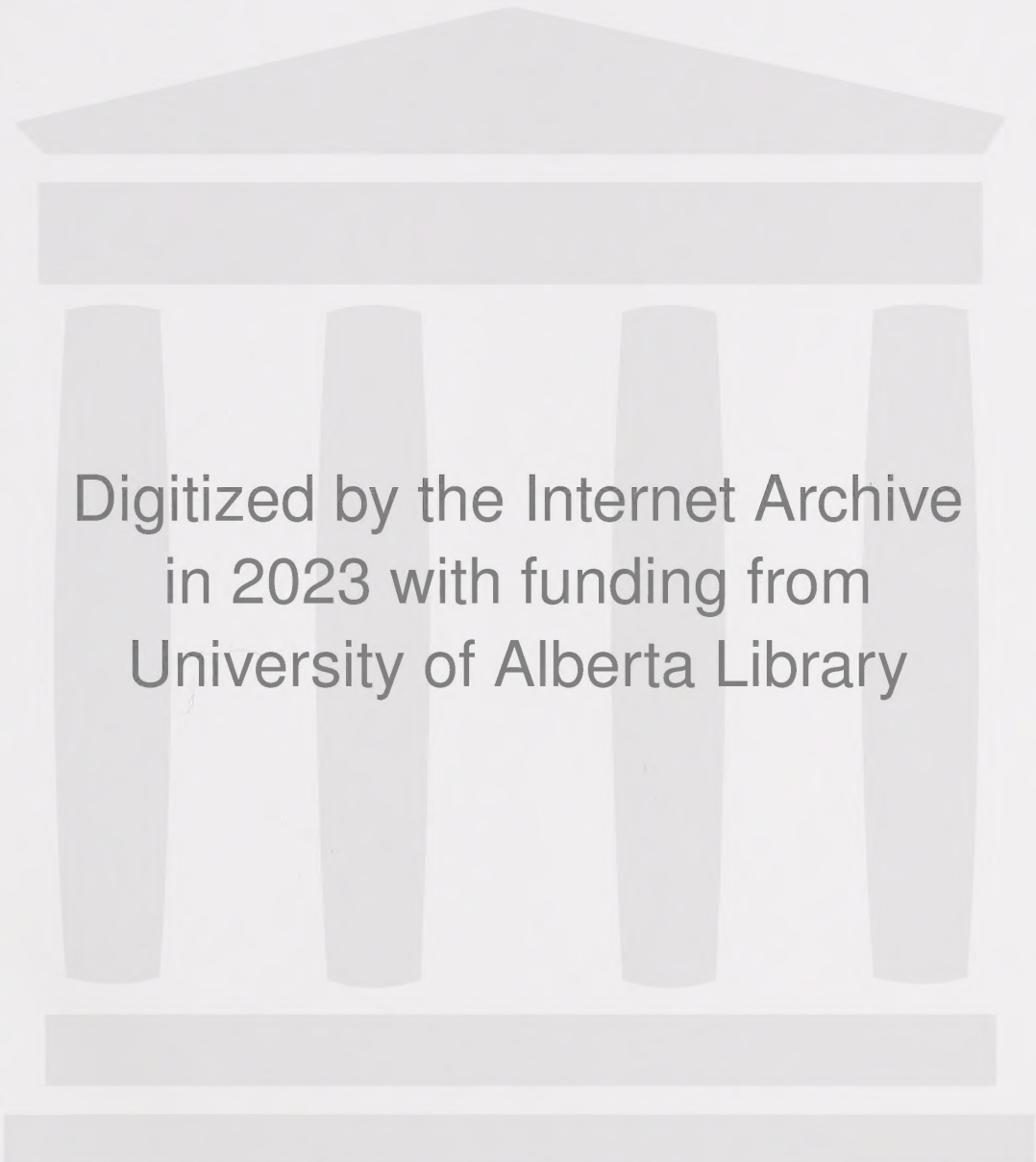

THE UNIVERSITY OF ALBERTA

THE RECOVERY OF PROTEINS AND LIPIDS FROM PACKING HOUSES WASTE WATERS

by

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A THESIS

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AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "THE RECOVERY OF PROTEINS AND LIPIDS FROM PACKING HOUSES WASTE WATERS" submitted by VLADISLAV MACHANDER in partial fulfilment of the requirements for the degree of Master of Science,

ABSTRACT

Slaughter house waste waters with a high organic content are usually disposed of by lagooning. Three types of ponds are used: anaerobic, facultative and aerobic. The major part of the effluent BOD is removed during waste residency in the anaerobic lagoon, primarily in the form of methane and hydrogen. The effluent from this pond flows into the secondary facultative and/or aerobic ponds before finally being discharged. This type of treatment of slaughter house waste waters is used in the city of Edmonton.

In many cases pretreatment of the waste is required, and the study presented is that of chemical pretreatment of the waters using dodecyl benzene sulfonic acid and sodium and ammonium salts of lignin sulfonic acid. For a comparison of the efficiency of oxygen demand index (ODI) removal by using these agents, the standard flocculant, aluminum sulfate (alum), was applied.

A comparison of ODI reduction and total solids removal revealed the lowest efficiency when the waste water was treated by standard procedure with aluminum sulfate, in which case total solids removal was 50 % for plant "B" waste water and 43 % for combined effluents. ODI reductions were 84 % and 68 %, respectively. By using dodecyl benzene sulfonic acid and salts of lignin sulfonic acid, the total solids removal was improved to 74 % for plant "B", and to 68 %

for combined effluents. The corresponding ODI reductions were 98 % and 95 %.

Based on the analysis of proteins and lipids present in waste water it was concluded, that the dodecyl benzene sulfonic acid and salts of lignin sulfonate flocculate the proteins near their iso-electric point with a simultaneous co-precipitation of lipids. The least efficiency in co-precipitation of lipids was obtained by standard alum treatment, in which case the amount of lipids was reduced from an initial value of 620 ppm to 590 ppm. More efficient was the precipitation with dodecyl benzene sulfonic acid (from an initial value of 620 ppm to 225 ppm), and the best results were obtained when lignin sulfonic salts were used (from 620 to 5 ppm).

An additional pollution problem created by application of chemical pretreatment of slaughter house waste waters has been considered. Based on a literature review, it appears that there are well defined biological mechanisms which are involved in biodegradation of the linear chain dodecyl benzene sulfonic acid and lignin sulfonates. Hence, the pretreatment of waste waters by the above mentioned methods should not create an additional pollution.

Finally, based on the literature review, it appears that the protein-lipid-dodecyl benzene sulfonic acid or protein-lipid-lignin sulfonate complexes might be as good as fish or soya meal proteins used as livestock feed. If an economical study were to prove

the feasibility of marketing of such by-products of slaughter house waste waters, the chemical pretreatment of such waste waters could offer not only reduction of existing trade effluent charges but also a substantial income from the sale of such by-products.

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ABBREVIATIONS USED AND GLOSSARY OF SOME TERMS

AAS	Atomic absorption spectrophotometry
ABS	Alkyl benzene sulfonic acid
Aerobic	A process or reaction involving or requiring the presence of the free oxygen
ALS	Ammonium lignin sulfonate
Anaerobic	A process or reaction not involving or requiring the presence of the free oxygen
Biodegradable	Being able to be assimilated by microorganisms
BOD	Biochemical oxygen demand. The amount of oxygen required for the biological oxidation of the organic matter in a liquid.
COD	Chemical oxygen demand. The amount of oxygen required for the chemical oxidation of the organic matter in a liquid
DBS	Dodecyl benzene sulfonic acid
GLC	Gas liquid chromatography
IG	Imperial gallon
IN	Iodine number. Represents the unsaturation degree of fat (oil) and is expressed in grams of iodine consumed by one hundered grams of fat (oil)
LAS	Linear, not branched, alkyl benzene sulfonic acid

Lipids	Animal or vegetable fats (oils) consisting of triglycerides phospholipids, sterols and similar lipophylic compounds
LSA	Lignin sulfonic acid
Metal elements	Ca, Mg, Na, K etc. usually present in g/100 level in waste water Fe, Cu, Co etc. usually present in ppm level in waste water
NMR	Nuclear magnetic resonance
ODI	Oxygen demand index. Under standardized procedure, the amount of dichromate consumed in wet ashing of organic constituents of waste water. Similar to chemical oxygen demand (COD) test
Potassium permanganate value	Earlier standard procedure for determination of COD. Due to inconsistent results it was replaced by COD test based on potassium dichromate
ppm	Parts per million by weight
Surfactants	Surface active agents, detergents, emulsifiers
TOC	Total organic carbon. The amount of CO_2 evolved from waste water sample after combustion in the stream of O_2 at 950°C

CHAPTER I

INTRODUCTION

1. Meat Packing Wastes

The population explosion and sweeping technological advances are urbanizing societies and creating unprecedented environmental degradation. The speed, magnitude and complexity of these forces intensify traditional problems. The gap widens not only between what is being done and what needs doing. In 1970 some 140 million cows, pigs and sheep were shipped to packing plants across the North American Continent and came out as steaks, bacon and lamb chops. The number that will make the same trip in 1972, according to the U.S. Department of Agriculture, could be 5 to 10 % higher.

Meat packing gives rise to vast quantities of waste water characterized by a high biological oxygen demand (BOD), offensive odor and a high suspended solids content (1). Blood, for example, has a BOD in excess of 150,000 parts per million (2), (3). A gallon of blood discharged into a sewer has the same oxygen demand as the untreated daily wastes of seven or eight people.

The meat industry is concerned with the problem of improving the quality of waste water effluents discharged into receiving streams. Although the challenge imposed by this problem is not

a new one, it is receiving greater emphasis at the present time because of the increased interest of the public in the quality of the environment and enforcement of more stringent water quality standards, at both the federal and provincial levels.

The meat industry is not unique in this regard, but rather shares a problem which also challenges other wet industries and municipalities. Improvement in the quality of meat industry waste water effluents infers further reduction in biochemical oxygen demand (BOD), grease (hexane solubles), suspended solids, and also reduction in compounds containing nitrogen and phosphorus because of the significance of these latter two elements in the biostimulation of the aquatic environment.

Because of the higher quality requirements for effluents and the resulting increased cost for waste treatment facilities, it is becoming increasingly important to improve in-plant housekeeping programs to prevent wastes from entering the sewage systems. This means that there is need to review cleanup methods.

2. Process for Removing Proteins and Decomposition Products from Waste Water

Effluents containing proteins, which may be more or less degraded into polypeptides, amino acids, and other nitrogenous substances during the processes which produce the effluent, are

discharged, not only from packing plants and slaughter houses, but from many other industrial plants such as dairies, fishmeal, corn starch, potato flour, cod liver oil and other processing plants.

Ordinary household waste can also contain large quantities of proteins, as well as fats and oils. The discharge of such untreated waste into natural receiving streams can cause problems since the waste has a high biological oxygen demand (BOD). This is because the degradation of the organic substances brought about by micro-organisms, such as bacteria, requires oxygen.

Large quantities of discharges of strong organic waste therefore make large demands on the oxygen in the receiving water, so that natural flora and fauna are deprived of their oxygen needs. Fish stock may be harmed and it is not unusual to have fish kills due to waste discharges.

The BOD of the waste can be often reduced by means of filters, either mechanical or the more efficient but costly biological filters. These work best, however, if the protein content, e.g. the content of organic or fixed nitrogen, is relatively low, since a high protein content, or organically fixed nitrogen, appears to retard the growth of bacteria on the biological filter. The same applies if, instead of using biological filters the effluent is treated by the activated sludge process or in lagoons (1),(4),(5).
It is thus important to remove proteins and similar substances from the effluent even if it is to be biologically treated.

3. Study Objectives

Based on the review of the conventional treatment processes used for treating meat plant waste waters, it is apparent that advanced treatment systems will be required to reduce proteins, lipids and other nutrients below the levels usually found in these effluents. Also, these valuable substances should be recovered for additional use in food industry (animal feed).

This study is related to a process for removing proteins and any decomposition products from the packing plant waste waters, and for the utilization of such products.

It has been found that, under acidic conditions with the aid of various chemicals, such as alkyl aryl sulfonic acid compounds or lignin sulfonates, proteins can be precipitated from waste more efficiently than is possible by conventional coagulation. The separated product, which consists of a complex of proteins, fats and their decomposition products with the precipitating agent, can, if required, be processed into livestock feed.

The goal of this study is:

(1) to determine the content of proteins and lipids in Edmonton packing plants waste waters;

(2) to determine the content of metal elements in such waters; and

(3) to verify the efficiency of new methods for removal of proteins and lipids from packing plants waste waters.

CHAPTER II

PACKING HOUSE OPERATION AND DESCRIPTION OF WASTES

1. Factors Affecting the Nature of Wastes

The waste from slaughtering and meat processing operation does not vary as widely in characteristics and concentration as might be expected from the wide range of processing operations and meat conservation practices found in that industry (TABLE B.1). There are however, no average plants and average conditions, therefore the figures in TABLE B.1 should not be used without a careful study. The strength, amount and nature of packing house wastes are governed considerably by such factors as:

1. general facilities of the plant and the type of operation being conducted, e.g. hogs, cattle, sheep

2. methods of killing

3. material handling equipment and methods

4. rendering facilities and methods

The size of the plant also affects the characteristics of the wastes as well as the volume. Very small slaughter houses may not save blood, and some do not provide grease recovery. Some small plants may wet-render but may not have facilities for evaporating the tank water, the liquid by-product of the process. The BOD of tank water will run as high as 30,000 ppm or more. Most rendering plants are now dry-rendering, a process which produces no tank water, but "skimmings" from grease recovery tanks are still generally wet-rendered because of the large amount of water in the "skimmings".

In the meat industry bacterial decomposition begins at the instant an animal is slaughtered and the bacteria usually grow according to a logarithmic curve (FIGURE II.1). To the packer this means rapid processing is necessary with the bacterial problem in mind at every step. Vast amounts of water are needed to avoid bacterial decomposition.

Measurements of water consumption in several packing houses with a mixed kill have shown the 24 hour volume of water to vary from 450 to 1,100 imperial gallons per animal or 360 to 800 IG per hog unit. (To convert animals killed to hog units killed a factor of 1.0 for hogs, sheeps, lambs, calves and 2.5 for cattle is used.)

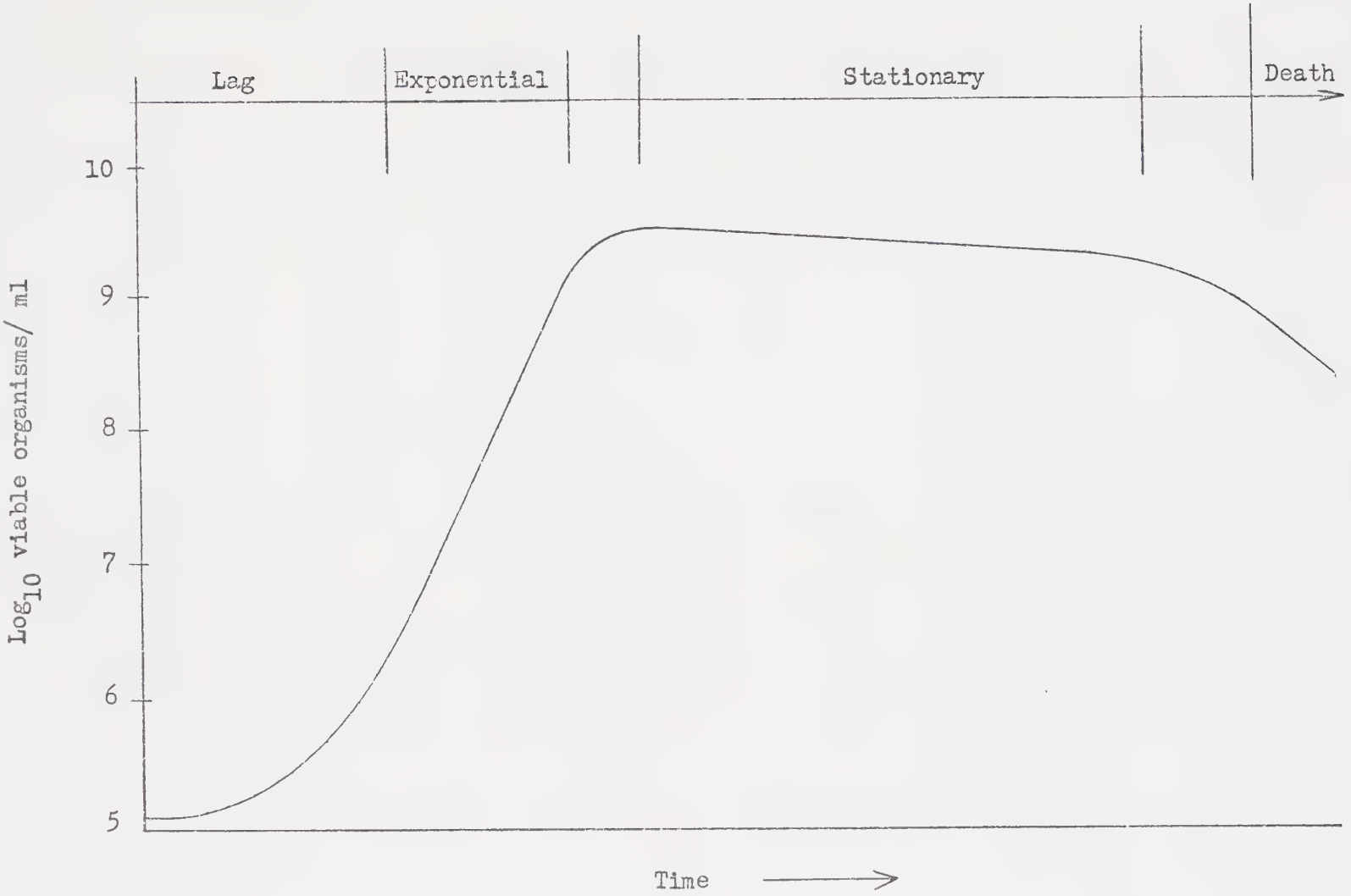


FIGURE II.1 TYPICAL GROWTH CURVE FOR A BACTERIAL POPULATION

2. Description of Wastes

(i) Fat Wastes

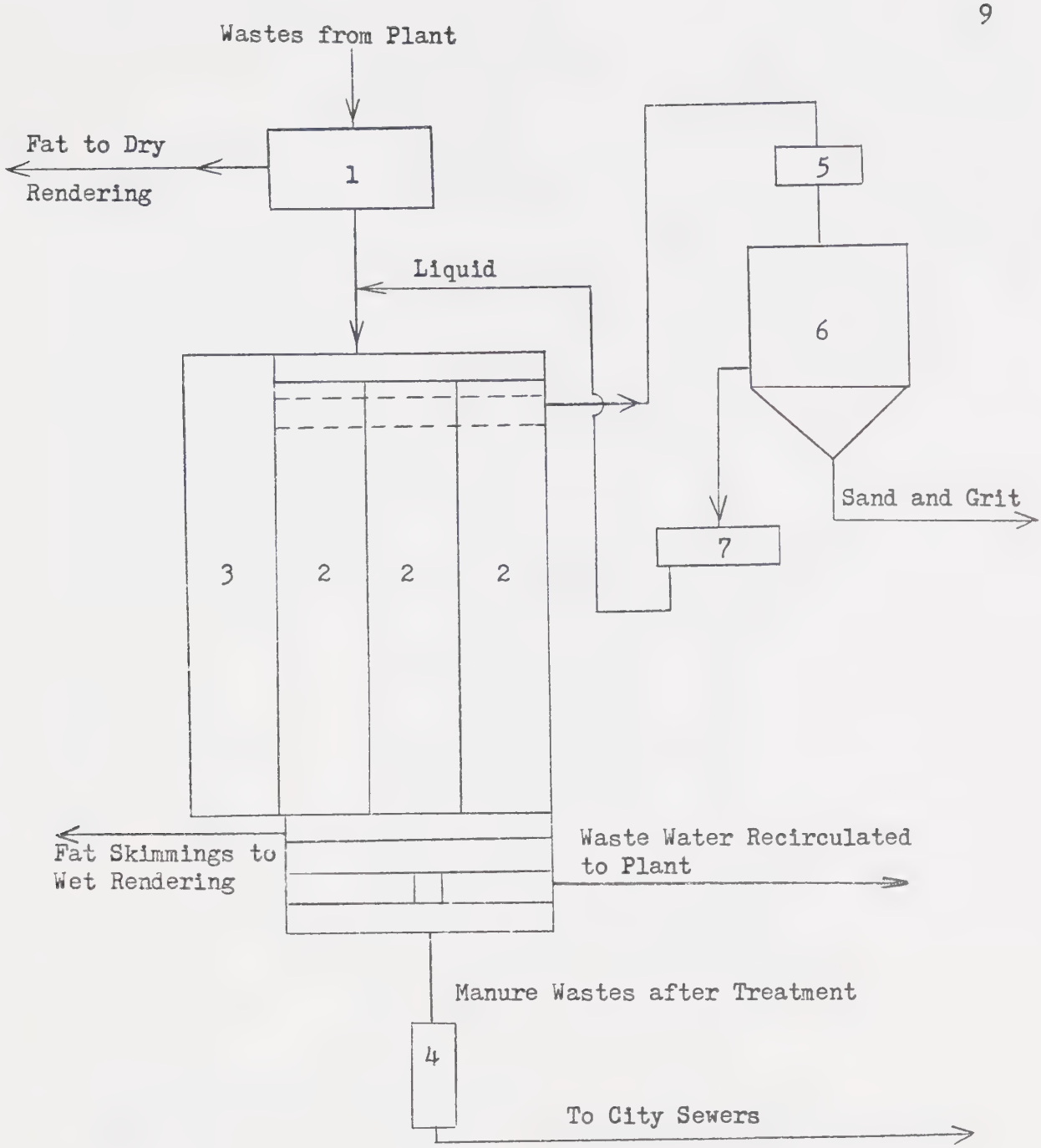
Fat wastes include water from departments such as: hog head boning, beef boning, pork cutting, rendering, casing after manure removal, fancy meats, cookroom, smoke houses, smoke meats hanging, lard manufacture, pre-packaging, curing, canning, ham boning, sliced meats and oil refining.

(ii) Manure Wastes

These include water from departments such as: hog scalding tank, hog dehairing machine, after removing hair and toes, stunning area, shackling pens, stock runways, pens, casing flushing, paunch opening if impractical to dump paunches directly to a screen or a truck, gut washers, tripe washer, hide cellar.

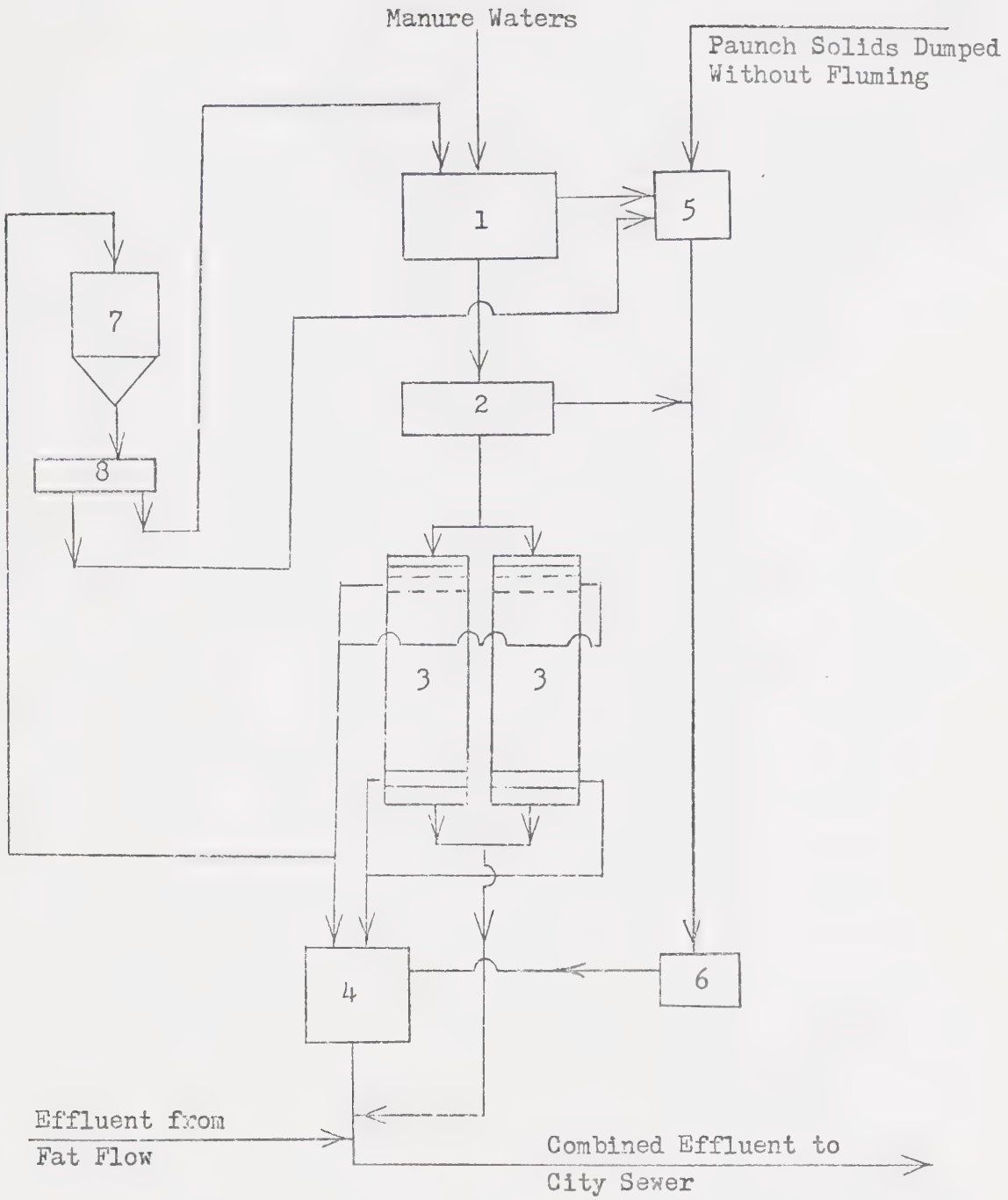
3. Waste Treatment Facilities

An example of an in-plant waste water treatment is a split flow plant built by the Canada Packers Ltd. at Winnipeg (FIGURE II.2 and FIGURE II.3).The flow diagram of this plant was presented by A.L. Van Luven at Sixth Ontario Industrial Waste Conference (Delawana Inn, Honey Harbour, Ontario, 1959).



1. Two 20-mesh selectro vib. screens 2. Three catch basins 3. Future air flotation tank 4. Automatic sampler 5. Hammer mill 6. Surge tank for sludge 7. Sharples Super-D-Canter.

FIGURE II.2 TREATMENT FOR FAT WASTES AT CANADA PACKERS IN WINNIPEG



1. Two 8-mesh selectro vibrating screens 2. Grit remover 3. Two catch basins 4. 20-mesh selectro vib. screen 5. Manure press 6. Garbage truck 7. Surge tank for sludge 8. Sharples Super-D-Canter.

FIGURE II.3 TREATMENT FOR MANURE WASTES AT CANADA PACKERS IN WINNIPEG

Fat and protein wastes are first passed over 20 mesh vibrating screens. The screenings are sent to the rendering plant and the liquid effluent is settled in three parallel operating tanks each 50 ft by 14 ft by 7 ft. These tanks provide a one hour retention period. The underflow is treated in a hammer mill before being collected in the sludge tank. This unit smooths out the feed to a Super-D-Canter which dewaters the sludge before final treatment in the blood dryer. After skimming of fats the liquid overflow is returned to the head of the plant. The Super-D-Canter has the capacity to discharge up to 3000 lbs/hr of wet solids. The resulting dry material from the bottom of the catch basins, when manure is excluded reasonably well, will analyse approximately as follows: Protein 30%, Fat 10%, Fibre 11%, Ash 13%, Carbohydrates etc. 36%. This material which is mixed wet with blood and dried is used for animal feeds.

The settling tank effluent is mixed with the effluent of the manure waste treatment unit. This combined flow goes to the city sewer via flow measuring and automatic sampling equipment. Manure water is screened on an 8 mesh vibrating unit with screenings going to the manure press. After screening the waste is treated in a grit remover, clarified in two parallel tanks and is then mixed with the fat and protein plant effluent. In the manure press, the screenings are mixed with paunch solids, dumped without fluming, and the sludge from the settling tanks which has been thickened in a Super-D-Canter. The liquid waste from the Super-D-Canter and the press is returned to

the influent of the plant. Solids from the press are mixed with the grit and the screenings from a 20 mesh vibrating screen, which receives the skimmings of the settling tank, and are collected in a truck for ultimate disposal. From the available data, it appears that the settling basins remove 65 to 95 % of the fat loading and 60 to 80 % of the suspended solids loading.

Different treatment facilities were found in Edmonton packing houses. See FIGURE II.4. At a slaughter house, which provides its own rendering (plant "A"), manure waste waters and waters from floor and equipment washing are mixed together and screened on a vibrating unit. The screenings are collected and used in a landfill. After screening the waste is treated in a catch basin with alum used as a coagulant. Floating grease is skimmed and collected in a grease tank. The sludge from the bottom of the catch basin is mixed with the waste influent before recirculation.

At packing plant "B" (FIGURE II.5), waste waters from the killing floor, containing raw materials (manure, paunch material, whole blood etc.), are screened on a vibrating screen and then treated in a paunch cell for grease removal by the help of aeration and chemical coagulation (Nalycolite 675). A similar process, expect the vibrating screen, is used for the wastes from the meat processing floor which are treated in a grease cell. The skimmed material, both from the grease cell and from the paunch cell, is heated in grease tanks and transported

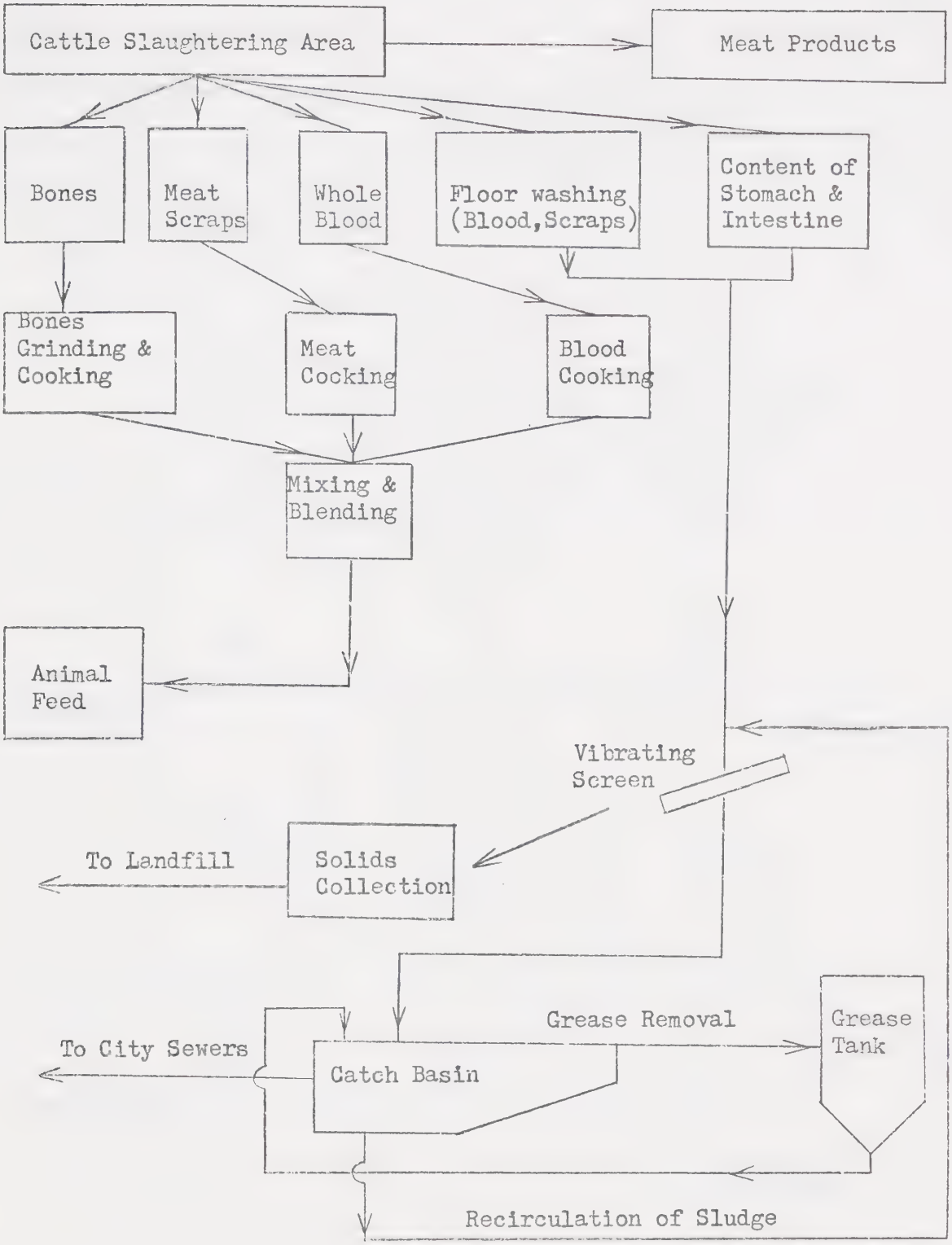


FIGURE II.4 TREATMENT OF WASTES AT PACKING PLANT "A"

to wet rendering by pumping. The waste waters from paunch and grease cells are mixed, and after passing through the flume and sampling device, discharged into the city sewer.

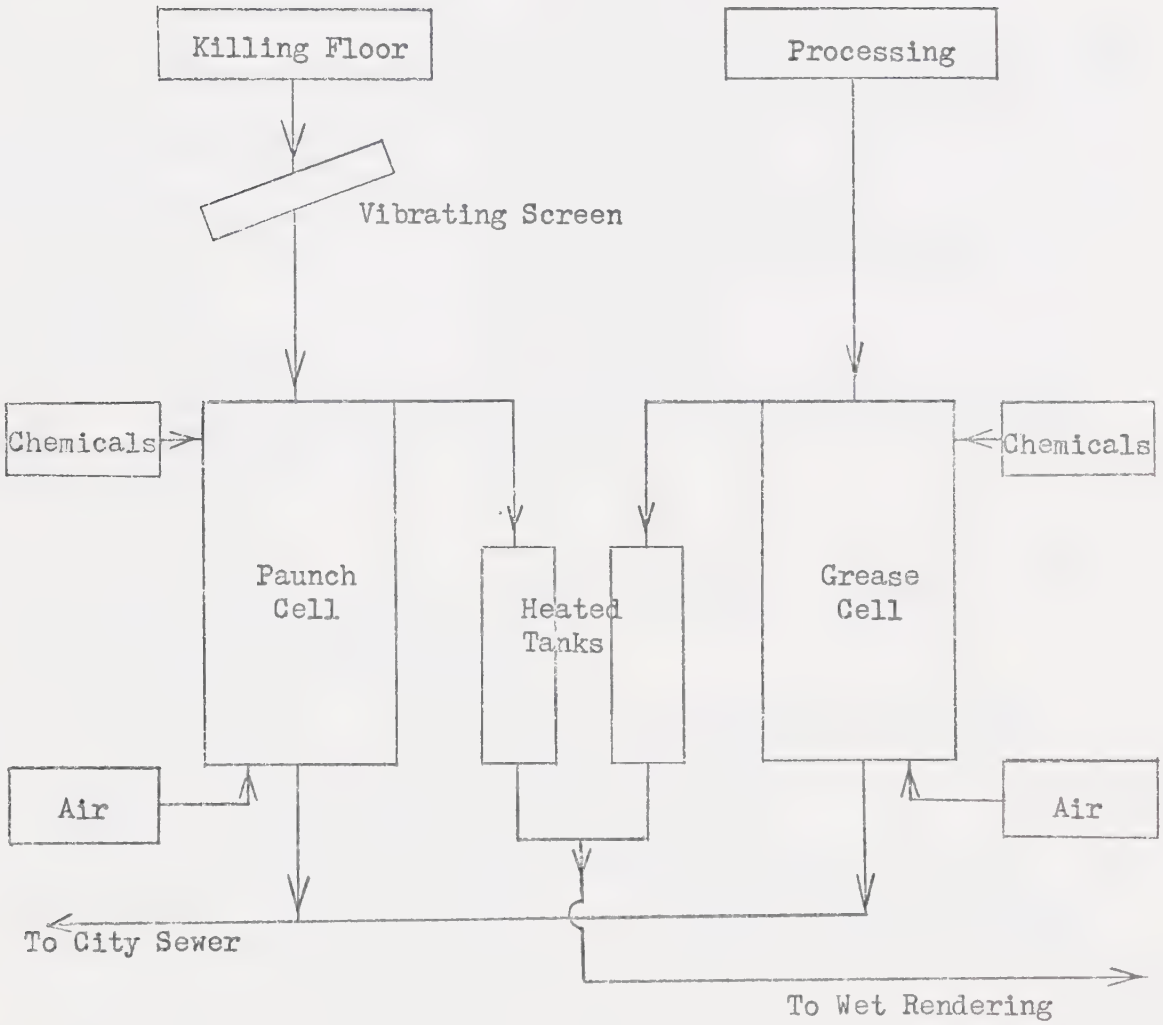


FIGURE II.5 TREATMENT OF WASTES AT PACKING PLANT "B"

CHAPTER III

TREATMENT METHODS FOR PLANT EFFLUENTS

1. Stabilization Basins or Lagoons

The popularity of stabilization basins for partial as well as complete treatment of meat industry wastes, has increased in recent years, and has been stimulated by developments in the pond treatment of municipal waste waters. Waste water ponds may be either aerobic or anaerobic or a combination of both types.

The following types, including both partial and complete treatment, are currently in active use:

i) Anaerobic (deep) ponds to reduce the strength of wastes prior to discharging to a municipal plant.

ii) Complete treatment in aerobic ponds, generally in series and preceded by good grease and solids recovery.

iii) Complete treatment in anaerobic-aerobic systems, in series, usually consisting of a single deep anaerobic pond, followed by one or more shallow aerobic ponds in series.

iv) Further treatment (tertiary) following anaerobic contact or conventional aerobic secondary treatment.

Anaerobic ponds are particularly useful in the packing industry because waste waters are generally warm and contain a high concentration of organic nutrients. Aerobic ponds rely upon algal growth and supply oxygen necessary to reduce BOD.

Primary anaerobic lagoon treatment followed by one or more aerobic ponds is used, particularly where large tracts of land are available at reasonable costs. It is estimated that BOD reductions with a combined anaerobic-aerobic lagoon system may be as high as 90 % under favorable conditions.

2. Activated Sludge Process

In this process waste water from which settleable solids have been largely removed is mixed with conventional activated sewage sludge. Although BOD reduction can be quite high under optimum circumstances (reduction as high as 95 % have been achieved), the high capital costs for the activated sludge plant make the process less desirable than lagoon systems for treating meat packing wastes.

3. Trickling Filters

As in conventional installations, aerobic organisms attached to a bed of filtering medium oxidize organic wastes, as the effluent trickles through the filter bed. BOD removals can be as high as 90 % with proper operation.

4. Anaerobic Contact

Anaerobic processes are particulary useful in the treatment of packing wastes and the anaerobic contact process is no exception(3). The system consists of an equalizer tank which prevents shock loads of pollutants from disturbing the balance of the anaerobic digesters, digester tanks seeded with anaerobic sludge organisms, a degasifier to remove dissolved methane, sulfur dioxide, and other odor-causing gases and to promote settling, and a settling basin (3), (41). Settled sludge from the basin is recirculated to the digesters until too much sludge has accumulated. Excess sludge is then trucked to agricultural fill. Anaerobic contact treatment is usually followed up with aerobic lagoons. BOD removal is in the neighborhood of 95 %.

5. Air Flotation

In the air flotation process a coagulating agent such as ferric chloride or alum is added to waste waters in a sealed treatment chamber, and air, under pressure, is introduced for a short period of time. With the iron salts acting as catalysts, the air apparently oxidizes organic components and aids in moving the floc to the top of the holding tank where it can be skimmed off. An average BOD reduction of 50 % can be achieved with air flotation techniques.

CHAPTER IV

TREATMENT OF WASTE WATER EFFLUENTS FROM PACKING HOUSES IN EDMONTON

1. Introduction

This chapter is based on research carried out by Professor P.H. Bouthillier from the University of Alberta and G. Brown from the City of Edmonton (6).

Prior to 1965, the City of Edmonton main sewage treatment plant was being operated at more than design loading in both the hydraulic and organic load sense. Packing house wastes constituted a large portion of the load and in addition contributed a large amount of grease. In order to reduce the load on the plant the wastes from the packing plants were routed to lagoons.

2. Location

The lagoons are located in a north easterly direction from the City of Edmonton. They lie on the right hand bank of the North Saskatchewan River. FIGURE B.1 and FIGURE B.2 show the main influent lines, the location of the lagoons on the north eastern outskirts of the City of Edmonton, and the sewer line serving the packing plants.

3. Function and Design

The lagoons were designed primarily to handle the waste from three packing plants (Swift's, Canada Packer's and Burn's). These waste flows are of the order of 2.5 mgd (Imperial gallons) with suspended solids of approximately 1,000 mg/l and five day twenty degree BOD of 1,000 to 2,000 mg/l. The packing houses have on their premises facilities for removal of fats, coarse material and paunch wastes. A critical factor in the design of the lagoons was the required reduction of waste load to the North Saskatchewan River during the winter months (November to May) when low flows combined with an ice cover create a condition which requires that minimal amounts of BOD should be released to the river. Lagooning of the wastes presented an attractive solution. Since total storage was provided it would eliminate from the river this entire waste load during the critical winter months. The use of facultative aerobic lagoons alone did not appear promising due to the heavy loading. The system installed is one in which facultative lagoons are preceeded by anaerobic ones. The layout and dimensions are shown in FIGURE B.3. The volume of the anaerobic lagoons is 8.2 million IG each at the operating depth of 20 feet, thus providing a theoretical detention period of 2 to 3 days, depending on the influent flow rate. The facultative lagoons are of sufficient volume to retain all influent flow from November to the following May, at a liquid depth of about 20 feet. The arrangement of piping at the lagoons is shown on FIGURE B.4.

Dual surface inlets and outlets are provided in the anaerobic lagoons. A central inlet-outlet is also present in each lagoon for purposes of sludge transfer. In addition a sludge recycle line is provided so that sludge may be drawn from the bottom of lagoons no.1 or no.2 and recycled to lagoon no.1. The influent flow into the lagoons was predominantely packing house wastes in the early years after the construction. A typical flow recorded in 1965 was follows:

Packing Plants	2.5 mgd
Beverly Sewage	0.26 mgd
Sherwood Sewage	0.40 mgd

Today (1972) the situation is quite different. The portion of packing house wastes is still about 2.50 mgd but the portion of domestic sewage from Beverley and Sherwood Park has increased. The total flow into the lagoons is now about 6 mgd.

4. Flow Pattern Used at the Lagoons

The design of the lagoons permits various flow patterns. The operating pattern, up to the year 1969, has been for the influent to flow into anaerobic lagoon no.1, the overflow from lagoon no.1 going to lagoon no.2. The effluent from lagoon no.2 was directed to one of the facultative lagoons. See FIGURE B.4.

The sludge from the bottom of lagoon no.1 was recycled back to the influent line and into lagoon no.1. On occasion recirculation

was from lagoon no.2 to lagoon no.1, but the frequency of this recycle (lagoon 2 to lagoon 1) was such that no real effect of sludge mixing could be attributed to that operation.

During the summer of 1971 the flow entering anaerobic lagoon no.1 was diverted to lagoon no.2 and the overflow from lagoon 2 was directed to lagoon 3. The purpose of this diversion was to clean-up the inlet pipes which were plugged with waste solids. After the clean-up the flow was again directed to its previous flow pattern.

5. Efficiency of Lagoons

The efficiencies of the anaerobic lagoons were found to be comparable with those of conventional lagoons treating domestic sewage (6). The facultative lagoons provide limited removal of pollutants. This is due primarily to the schedule of operation which requires that the facultative lagoons be drained in June of each year when river flows are of the order of ten to twenty thousand c.f.s. compared to winter flows of the order of two thousand c.f.s. under ice cover. During the critical winter period the lagoon installation is one hundered percent effective - since there is no flow to the river. There is no attempt to use these lagoons as treatment units, other than by detention. The facultative lagoons are twenty feet in depth, therefore the surface to volume ratio is such that algal growth and surface aeration are not important factors in their operation. They are drained shortly after

the ice has melted off. The efficiencies established in 1968 are as follows:

i) Overall efficiency:

BOD removal	95%
Grease removal	95%
Suspended solids removal	90%

ii) Anaerobic lagoons efficiency:

BOD removal	88%
Grease removal	92%
Suspended solids removal	59%

6. Scum and Sludge Accumulation

During the four years of operation (1965 - 1969) a dense scum layer was created on the surface of the lagoons, specially on the lagoon no. 1. The depth of this layer at the thickest point was measured in the year 1970. It was found to be about 7 feet thick (2 feet above the liquid level and 5 feet below). The overall density should be thus about 5/7 of that of water.

The composition varied from two percent to a high of eighteen percent of grease. An average was found to be about ten percent. Very little floatable fat passed into lagoon number 2. This large volume of fat represents a possible reclaimable product, but the first objective of the City is to control the scum so that it does not unnecessarily reduce lagoon volume. As a trial measure the fat layer was ignited in the fall of 1966. Ignition was done with the aid of diesel fuel. Combustion was self sustaining until the fall of 1968. There was a considerable amount of smoke created for the first few days of burning and an odor of burning fat was present. The presence of a scum layer is not quite undesirable, because it helps to create better anaerobic conditions.

The primary problem of the lagoons seems to be sludge accumulation. The useful life of the anaerobic lagoons will be determined by the net volume remaining for settling of sludge. A detailed survey and analysis of lagoons no.1 and no.2 was carried out by the City of Edmonton since the year 1965 to 1969 (6). Some attempts were made to determine a sludge level. A photoelectric cell and a depth sounder were tried without succes. It appears that while there is likely a sludge boundary it is not sharply defined.

7. Conclusions

The anaerobic lagoons were initially designed for a flow of 2.5 mgd and detention period of six days. Due to the increased flow and

sludge accumulation this time is now substantially reduced to about 2 days. Unfortunately, there are no new data available for establishing the efficiencies of the tratment process with increased flow (6 mgd).

The flow pattern into the anaerobic lagoons has been changed several times and there will be probably more changes in the near future. But it seems highly probable that the existing system will have to be improved in order to guarantee sufficient treatment.

CHAPTER V

THE ANALYSIS OF WASTE WATERS FROM PACKING HOUSES IN EDMONTON

1. Purpose

This study was undertaken to determine the amount of lipids, proteins and mineral components in waste water effluents from packing plants. A second objective was to determine the strength of wastes (polluting capacity). For this purpose the Biochemical Oxygen Demand (BOD), Total Organic Carbon (TOC) and Oxygen Demand Index were also assessed on a number of samples.

2. Sampling Procedures

Three types of samples were used in this study for various analysis:

a) 24 hrs composite samples of the combined effluents of three Edmonton located packing plants "A", "B", and "C". These plants discharge their waste waters into Edmonton City Sewer System which directs them into the treatment lagoons. The samples were taken once or twice a month and used for following tests:

i) Total Solids

ii) Mineral Composition

iii) Proteins

iv) Lipids

v) Total Organic Carbon

vi) Biochemical Oxygen Demand

vii) Oxygen Demand Index

b) Combined samples containing a mixture of the above mentioned three effluents. These samples were taken several times from a city manhole and tested for:

i) Total Solids

ii) Proteins

iii) Lipids

iv) Mineral Composition

c) Samples from packing house "B". These samples were taken from the waste water line between the vibrating screen and the paunch cell, see FIGURE II.5. The samples were taken every fourteen days and tested for:

i) Total Solids

ii) Oxygen Demand Index

3. Description of Analytical Procedures

i) Total Solids

About 1 liter volume samples were taken for the determination of total solids. Well mixed samples (electric mixer was used) were evaporated on a water bath and then dried at 103°C for one hour. They were then allowed to cool to room temperature in a desiccator before weighing (7). The results expressed in mg/l (ppm) have been calculated from the following formula:

$$\text{mg/l Total Solids} = \frac{\text{mg Total Solids} \times 1{,}000}{\text{ml Sample}}$$

ii) Mineral Composition

One liter of waste water was evaporated to dryness on a boiling water bath. The residue was extracted by hexane in order to remove lipids. The defatted residue was divided into two portions, for protein determination and for mineral composition. The second portion was ashed by acid digestion using mixture of nitric and perchloric acids.

The ashing was performed in a laboratory beaker on a hot plate at 180°C for two hours, at which time the organic matter was ashed. The remaining mineral constituents (in form of soluble chloride salts)

were dilluted with distilled-demineralized water and used for aspiration in a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer (AAS).

Before the sample was tested for sodium, potassium, calcium, magnesium, iron and copper contents , the calibration curves for each element were constructed, using standard solutions of known concentrations (Appendix C). The results for the samples were read out from the calibration curves.

iii) Proteins

The Micro-Kjeldahl method was used for the determination of organic nitrogen and protein contents of waste water samples (8). After evaporation and hexane extraction a portion of a residue, in a quantity greater than 10 mg and less than 100 mg was tested. The complete procedure is described in Appendix G.

iv) Lipids

After evaporation of one liter of waste water the brown colored residual was extracted with normal hexane in a Soxhlet extractor for six hours (9). The crude lipid extracted was then further analyzed for iodine value by Nuclear Magnetic Spectrophotometer 303, and for fatty acid composition. Iodine value was determined by nuclear magnetic resonance. The procedure used was essentially that recommended by Varian Associates

(Shoolery J.N., High Resolution Nuclear Magnetic Resonance, Varian Associates, 1968). Data and calculation results for nuclear magnetic resonance are given in Appendix E.

Fatty acid composition of lipids was determined by gas liquid chromatography. Using a 5 ml round bottom flask equiped with an air-condenser, 0.1 g of crude brown colored lipid, in presence of 2 ml of absolute methanol, plus half a drop of concentrated sulfuric acid, was gently refluxed at 70°C for three hours. The reaction mixture was then washed with water, extracted with n-hexane and finally washed with potassium bicarbonate, dried with sodium sulfate and concentrated to a volume of 1 ml by a stream of nitrogen. This solution was then used for determination of fatty acid composition by straight injection into a gas liquid chromatograph.

v) Total Organic Carbon

The total organic carbon test (TOC) was run on a Beckman Model 915 Carbon Analyzer by the Laboratory of the Department of Public Health. 20 ml blended samples were supplied to the Laboratory for testing. The procedure involved combustion of the sample in an electric furnace in a stream of oxygen. The resulting carbon dioxide was measured in a nondispersive infrared analyzer and the results were recorded as mg/l of carbon on a strip chart. A flow diagram of this process is shown on FIGURE V.1.

The problem of inorganic carbon, i.e. carbonates and carbon dioxide present in the sample is solved by the use of two combustion chambers, one maintained at 950°C to oxidize total carbon, the other at 150°C to oxidize inorganic carbon only. Two runs are required, using one portion of the sample in each furnace. The total organic carbon concentration is obtained by the difference between the total carbon and the total inorganic carbon peaks on the recorder (10).

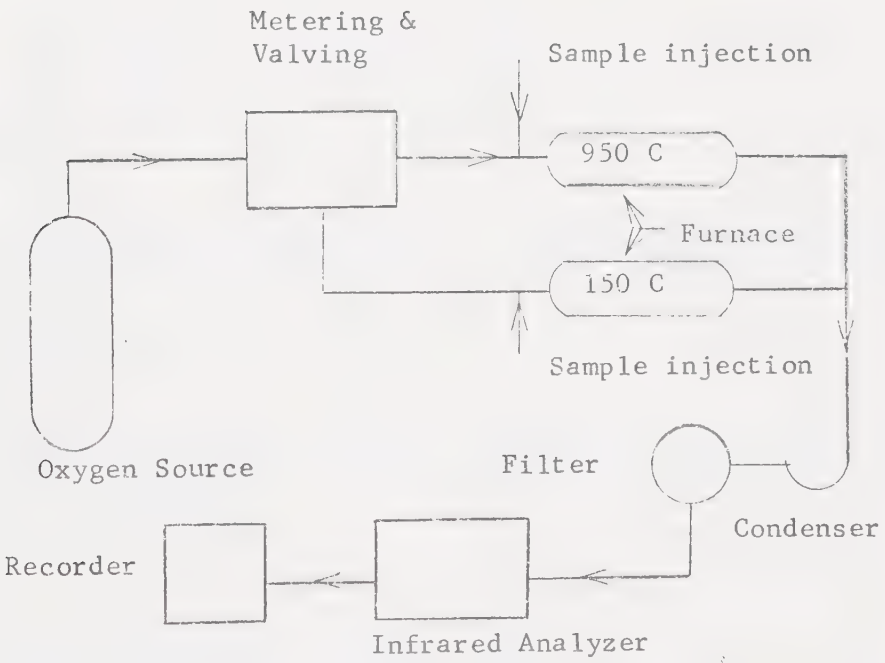


FIGURE V.1 FLOW DIAGRAM OF TOTAL CARBON ANALYZER

vi) Biochemical Oxygen Demand

This test was run at Edmonton Main Sewage Treatment Plant. The details of the method are described in Standard Methods for the Examination of Water and Wastewater (11). Instead of duplicate samples required by standard procedure, triplicate samples were used with 0.25 ml, 0.50 ml and 1.00 ml of sewage (before dilution). The calculation of results was done by using the formula given below and the resulting BOD values of triplicate samples were averaged.

$$\text{BOD mg/l} = \frac{D_1 - D_2}{P}$$

D_1 = Dissolved oxygen of diluted triplicate sample 15 minutes after preparation.

D_2 = Dissolved oxygen of diluted triplicate sample after 5 days $20^{\circ}C$ incubation.

P = Decimal fraction of sample used.

vii) Oxygen Demand Index

The oxygen demand index (ODI) is a chemical test which uses potassium dichromate to oxidize the organic matter in a sample of waste.

A color change of dichromate from yellow hexavalent to green trivalent is proportional to the organic matter in the sample and can be related to oxygen demand. Results are obtained in approximately 30 minutes which is a great advantage in comparison to time necessary for BOD test (5 days). The procedure for the oxygen demand index is given in Appendix D.

CHAPTER VI

COAGULATION EXPERIMENTS

1. Coagulation

In the coagulation tests a sample of 600 ml of waste water was transfered into a 1,000 ml beaker under a mechanical stirring device (Phipps & Bird, Inc. Laboratory Stirrer, Model 77 - 903).

The amount of sulfuric acid necessary to lower pH of the sample to a desired level and coagulation aids were added rapidly. The content of the beaker was then stirred vigorously for 3 minutes (80 rpm) followed by a 20 minutes slow mix (20 rpm). Solids were then allowed to settle for 20 minutes. Supernatant liquor was collected and tested for the amount of total solids and for ODI. The coagulation procedure was repeated with various doses of chemicals at different pH and temperature ranges. A list of chemicals used for coagulation is given in Appendix F.

2. Sulfuric Acid Consumption for pH Control

In order to determine the required acid dosage a 100 ml sample of combined treated sewage from three Edmonton packing plants was placed

in a beaker. The temperature of the sample was kept at 20°C. 0.1 normal solution of sulfuric acid was slowly pipetted to the sample. A vigorous stirring was provided by a magnetic stirrer and continuous change of pH was measured by Zeromatic II, Beckman pH-Meter.

CHAPTER VII

DISCUSSION OF COAGULATION METHODS

1. Processes Investigated

In the recent years, as an alternative to biological treatment systems, chemical processes have been developed and recommended for selective precipitation of organic compounds present in waste waters. That has been especially true in manufacturing and processing of foods of animal and vegetable origins. Usually, these processing units, before discharging their waste waters into rivers and/or sewers, carried out some form of a pretreatment, among which the pH adjustment was one.

Physical processes have been also employed, especially in the case of slaughter houses, oil seed and margarin production units, where the removal of fats by flotation or by emulsification was often necessary.

Chemical pretreatment of the waste waters is based on the use of either detergents or certain lignin sulfonic acids, both of which have the property of combining with proteins, thus promoting their flocculation. As found by Jantzen (1) the lignin sulfonic acid molecules of high molecular weight have the ability to precipitate the protein. For this purpose, each lignin molecule has to carry a sulfonic acid group, before it would function as a precipitant. Based on Jantzen

findings a commercial process was developed by Statens Technologiske Institut, Copenhagen (35). This process is a subject of a number of patents in various countries (1),(4),(5). The essence of the process is to convert the protein from waste water into a saleable animal feed by-product, as well as to reduce greatly the BOD of the water.

Considerable information exists for the use of recovered protein materials for feeding purposes. Toxicity tests and protein conversion factors have shown that proteins with lignin sulfonic acid are suitable as livestock feed (35). Tonseth and Berridge (35) studying the protein removal from industrial waste waters found that there is a difference in treating the waste water with pure lignin sulfonic acid and lignin sulfonic acid containing sulfite lye. Using the pure lignin sulfonic acid the BOD of the waste water was reduced more efficiently than with raw sulfite lye. As suggested by these authors the lower efficiency of raw sulfite lye is likely due to the presence of wood sugars and other impurities, which can contribute to the organic load.

Analytical data have been reported for a wide range of waste materials treated with pure lignin sulfonic acid. These include slaughter houses, fish meal and filleting factories, bone and pet food manufacturing plants and potato processing units. Except for the potato processing units the pure lignin sulfonic acid was able to reduce the BOD by 70 to 90 %. For treating the slaughter house wastes in a pilot plant it was found that the total solids content was reduced from 4,064 mg/l to 2,932 mg/l while the BOD was reduced from 1,015 mg/l to 253 mg/l (35).

By comparison, under the same conditions, the sulfite lye decreased the total solids from 3,068 mg/l to 2,848 mg/l and the corresponding BOD reduction was from 927 mg/l to 455 mg/l.

In the pilot plant the influent was first acidified to overcome the natural buffering capacity of the proteins present. Usually, this acidification involved the pH adjustment to 6.0 by adding sulfuric acid. The initial acidification was the followed by additional adjustment of pH to 3.5 by adding lignin sulfonic acid. The value of pH 3.5 corresponds roughly to the isoelectric point of the animal protein present in waste. Hence, the buffering capacity of the protein was actually saturated by sulfuric acid while lignin sulfonic was used for final achievment of the isoelectric point, followed by the subsequent complex forming reaction. After chemical treatment in the pilot plant the acidified waste was discharged to an air-flotation unit, where a solid-liquid separation took place. The concentration of the solids skimmed during flotation varied between 3-6 %. As suggested by Tonseth and Berridge (35), such a sludge could be readily further concentrated in a centrifuge or on a filter, and then finally dried by any conventional method.

The use of dodecyl benzene sulfonic acid (DBS) for removal of proteins from waste water is based on an observation patented recently in Britain (1). An efficient removal of proteins from waste water is feasible by simple precipitation when an aryl or aryl-alkyl sulfonic acid, or corresponding sulfonate, is used with the aryl moiety being a benzene or naphtalene ring. In addition, the patent claims that an efficient

flocculant should have alkyl groups, consisting of eight to twenty carbon atoms, attached to the aryl moeity.

The separated product, which is not well defined, could be a complex of proteins and/or their decomposition products along with the precipitating agent. This product could be processed further into livestock feed. The use of this process for protein precipitation does not include the removal of fat and oil. Hence, according to this patent the fat portion of the waste water has to be removed before precipitation by the conventional means, such as scraping, flotation or centrifugation. As stated in the same patent, the precipitation is most effective when performed at a pH between 3.0 and 4.5. It was also recommended that the precipitating agent should be added together with a strong acid, specifically nitric or sulfuric acid. Using protein rich waste, consisting of blood albumin, it was found that the efficiency of the DBS precipitation is comparable with that of sodium lignin sulfonate. The total clarification expressed as potassium permanganate value of the filtrate was better by 23 % using DBS instead of lignin sulfonate. In an attempt to explain the difference in clarification effect between these precipitating agents the patent suggests that the protein molecules can react with more molecules of DBS than with sodium lignin sulfonate. In addition, it was suggested that the DBS precipitate certain amino acids which cannot be precipitated by sodium lignin sulfonate. To explain the differences between these precipitating agents it has to be assumed that the solubility product values are different for DBS and sodium lignin

sulfonate. In the following equation

$$L = K_{prot}\ K_p^n$$

where:

L = solubility product (solubility constant)

K_{prot} = concentration of protein (g/l of waste water)

K_p^n = concentration of precipitation agent (g/l of waste water)
with "n" representing the number of molecules of the agent
binding with each protein molecule

Hence, L value of sodium lignin sulfonate ($L_1 = K_{prot}\ K_{ls}^n$) is less than the value of dodecyl benzene sulfonic acid ($L_2 = K_{prot}\ K_{dbs}^n$). On the other hand, the assumption cannot be excluded that the protein-DBS complex reacts further with the excess of DBS molecules forming a DBS enriched complex.

$$\text{Prot-DBS}_n + \text{DBS} = \text{Prot-DBS}_{n+1}$$

As a consequence, the BOD of the supernatant liquor should be lower. The precipitate separated should be a protein enriched sediment which can be used as livestock feed.

There is also a suggestion that protein enriched sediment could be applied as an adhesive for pelleting other animal feed. In that case it would be a cheap digestable and nutritionally rich protein glue. If the protein enriched substance is intended to be used as livestock feed by itself, then an additional pH value adjustment would be necessary. From a pH of 3.5 it should be increased to a value of pH 6.0-8.0. Such a substance then could be further processed into a livestock feed powder by any conventional drying method. The spray drying process is the one usually suggested, since the particle size obtained by spraying eliminates the need of additional grinding.

2. Alwatech Process - a Review of Patent Claims

The Alwatech process introduced recently in Norway, Sweden and Britain (36) is essentially a process based on precipitation of proteins and fats from waste water with purified lignin sulfonate near the isoelectric point of the proteins. For this reason adjustment of pH to 3.0, by the addition of sulfuric acid, is recommended. The protein precipitate formed readily flocculates and the fat particles present in the are practically co-precipitated with flocs. According to Alwatech process the flocs are not separated by gravity sedimentation. Floc separation is obtained by airflotation since the flocs have a specific gravity similar to water. Using a conventional tank the flocks rise to the surface of the tank where they are removed by scraper, skimmer or

by suction. The Alwatech process claims a solids concentration of 5.0 to 15.0 % of proteins and occluded fats is achieved. After solids removal the remaining liquor is discharged into a neutralization tank to readjust the pH to 7-8. The neutralization is carried out by lime slurry. Alwatech process, as described is recommended for treatment of waste water of slaughter houses and poultry packing units. The Alwatech process is able to remove 70 to 90 % of the BOD from a slaughter house waste waters. In this removal most of the protein nitrogen is involved. After the neutralization, the BOD found in the effluent is less than 20 mg/l and as such the waste water can be discharged to sewers. With the reduction of BOD there was an appreciable reduction of fat content.

If the waste waters are rich in fat contaminants, as it would be the case in margarine or oil seed factories, the air flotation is able to reduce the fat content from 1,250 to 464 mg/l (36). When the separation is achieved with pretreatment of the waste water with aluminum sulfate and lime at pH 7.0 a further dicrease of fat content can be achieved and the effluent fat then amounts to 56 mg/l. As claimed by Alwatech process, a similar reduction of fat in the effluent can be obtained when the fat is mixed with protein, and as a mixture precipitated by pure sodium lignin sulfonate.

From recent pilot scale tests at a slaughter house waste water treatment plant in Britain (36), the waste water BOD before treatment was 1,791 mg/l which was reduced to 217 mg/l. The BOD value can be further reduced by conventional activated sludge process or by biological filtration.

The sludge obtained by Alwatech process is actually a protein concentrate containing variable amounts of co-precipitated fats. It also contains soluble mineral components such as salt and trace elements. The amino acid composition of the proteins precipitated with sodium lignin sulfonate has also been reported. As compared to untreated proteins, no change in composition has been found. Bioassays and practical feeding trials on poultry (37),(38),(39), demonstrated that the protein concentrate complex with sodium lignin sulfonate is as good as fish or soya meal protein. Hence, the Alwatech process offers an increased income from the sale of by-procucts while at the same time offers the reduction of trade effluent charges.

3. Lignin Sulfonic Acid

Lignin sulfonic acids are by-products from the sulfite pulping of wood. The pulping industry in Canada is one of the leading industries not only in the country but also in the world. When the lignin sulfonates are discharged into lakes and rivers they create pollution problems.

The suggestion to use lignin sulfonates for purification of waste waters from slaughter houses are based on the experimental results which show an efficient precipitation of the proteins and protein-fat particles by adding these sulfonates in amounts ranging from 50 to 300 ppm. An excess of lignosulfonates in the remaining supernatant liquor may create an additional pollution problem. Earlier reports

indicated an extreme resistance of lignin sulfonates to microorganisms. However, recent reports defined a number of biological mechanisms which may be involved in the decomposition of lignin (12-19). A brief consideration of the nature of lignin itself and of lignin sulfonates and their responce toward microorganisms, as a possible way of biodegradation of these compounds, should therefore be elaborated.

Kleinert and Joyce (18) obtained the evidence of the utilization of lignin sulfonates by mixed cultures of fungi and bacteria. A variety of fungi were used by Ledingham and Adams (20) to evaluate deconposition of calcium lignin sulfonate. Maximum utilization was 12 to 18 % in two months. Abernathy and Watson (22) found that a bacterially enriched culture utilized lignin sulfonates to the extent of 10 % in 21 days. Other authors (23) have shown that fungi capable of growing on native lignin do not grow on lignin sulfonates.

Although the data are somewhat conflicting it appears that lignin sulfonates are more resistant than lignin to biological decomposition. Watkins (21) claims that there are two reactions which lignin undergoes in the sulfite process:

1) Sulfonation of side chains of aromatic monomers.
2) Hydrolysis of ether linkages and the formation of new carbon-carbon bonds between aromatic rings.

These reactions might increase the biological resistance of lignin.

As proved by Watkins (21) in growth studies with model compounds, which constitute the structure of lignin, and soil bacteria there is a relationship between the molecular structure and availability

to microorganisms. All of the compounds which supported good bacterial growth had side chains with primary alcohol, aldehyde or carboxylic functional groups. (Vanillyl alcohol, cinnamyl alcohol, hydrocinnamyl alcohol, ferulic acid, alpha conidendrin etc.). On the other hand, the presence of methyl group or sulfonate substitution in the side chains reduced the biodegradability. (Vanillyl sulfonate, acetovanillone). Another possibility was that sulfate reducing bacteria might be able to reduce sulfonate groups thereby making sulfonated aromatics more available. The cultures tested were Desulfovibria from river mud and Clostridia predominantly from garden soil. Watkins (21) found that the mixture of these bacteria did not increase the availability of vanillyl sulfonate to aerobic soil bacteria.

Additional studies using lignin sulfonates and bacteria from river water, showed that unlike soil bacteria, they grow well on the sulfonated monomers. (Vanillyl sulfonate and vinylvanillyl sulfonate). In addition, the type of bonding between monomers had an effect on availability of dimers. Those with bonding between side chain carbons, as in vanillyl alcohol, or aryl-alkyl bonds, as in alpha conidendrin, were readily utilized for growth. FIGURE B.5. As found by Watkins (21), using vanillyl sulfonate as substrate, the sulfonate could be degraded completely by river bacteria when initial concentrations of the substrate were 100 - 1,000 mg/l. The optimum pH for decomposition was found to be 7.0 while at pH 8.0 activity decreased to 50 % and at the acidic pH of 5.0 it was inhibited completely.

As stated in Appendix F of this report Orzan A, used as a flocculant, is a spray-dried lignin extract consisting of 57 % of lignin sulfonic acids, 17 to 20 % of wood sugars and approximately of 3.7 % of nitrogen in the form of ammonia. For the study of biodegradability, this product was fractionated on a silica-gel column into three portions which were further tested as substrates for river water bacteria (21). All three fractions supported good bacterial growth for three or more days. In addition, these experiments also provided a suggestion that the substrates collected from the gel column were selective for different species or strains of bacteria present in the mixed culture used as inoculum.

With the lignin sulfonate fraction containing high molecular weight compounds a long lag period followed by rapid growth suggested a bacterial adaptation to a new substrate.

From all these experiments it appears that the suggestion to use lignin sulfonates for waste water treatment would not cause an additional pollution problem. Moreover, the protein-fat-lignin sulfonate complex can be additionally pelleted and used as livestock feed (24). A lignin extract is generally recognized by animal nutrition experts as safe for use at levels not exceeding 3 % when applied as a binder for animal feed pellets.

4. Dodecyl Benzene Sulfonic Acid

In experiments conducted in this study dodecyl benzene sulfonic acid (DBS) also effectively precipitated proteins from waste waters. If the use of DBS is accepted then the slight excess of DBS left in the supernatant liquor might induce an additional pollution. Such pollution is actually dependant on the biodegradability of DBS.

The question of nonbiodegradability of surfactants in general has been emphasized and has been a contraversial point in recent years. All of these chemical compounds which have been manufactured are in fact biodegradable. The paramount issue involved is the rate of degradation of the molecule and the corresponding loss of the molecule's surface activity (25).

Based on experimental results it is generally agreed that straight chain (aliphatic) compounds are subject to faster and more complete degradation than branched chain or aromatic group compounds (26-29).

A straight chain ABS was shown to degrade 95 % in 12 days (30). The major change from ABS to LAS by the chemical industry has involved the change in the alkyl group from a tetrapropylene (branched) to a linear (non-branched) chain. A multitude of possibilities exists for chain length in the alkyl group, points of attachment on the benzene ring, and ethylene oxide chain length. Allred and Huddleston (31) tested several compounds of varying molecular structure using the river die-away test Their data showed LAS to degrade to the extent of 100 % in 5 days and

a nonylphenol ethoxylate to degrade only 54 % in 30 days. Comparatively higher rates were quoted by Brink and Meyers (27). They demonstrated that certain alkyl sulfates degraded from 96 to 97 % in 25 hrs and a straight chain ABS degraded 94 % in the same period.

From the data obtained by Davis (25) it can be assumed that algae and associated bacteria contribute to a small extent in the degradation of the surface active agents. Most of the degradation is attributed to bacteria and other microorganisms found in waste water environments.

According to Bock (32) only such anionic detergents which have at least a biodegradability of 80 % are now permitted in most countries. Bock (32) also states that the tetrapropylene benzene sulfonate containing a branched alkyl chain is degradable to 30 % while the straight alkyl chain is degradable to an extent of 95 %. The decomposition of straight chain ABS during purification of sewage is so rapid and complete that even greater amounts than those usually found in sewage treatment plants can be biologically degraded.

Truesdale and Eden (33) state that synthetic detergent powders introduced in Great Britain on a large scale in 1948, were based mainly on the biologically resistant sodium tetrapropylene benzene sulfonate. Due to their resistance they are now replaced by the so called soft detergents which are linear ABS and are more readily decomposed by micro-organisms.

For the biologically resistant ABS treatment, R. Samples from the California Institute of Technology (34) suggested the use of cationic

detergents to precipitate anionic detergents by forming the slightly soluble anionic-cationic complex. As stated by him the cost of this treatment is as competetive as other proposed methods for the removal of anionic detergents, such as Jantzen filter (1). However, Sample's procedure may not be any more justified for the removal of ABS from waste waters because of the introduction of new biodegradable products (25).

Since nowadays DBS type of detergents are exclusively manufactured from the group of linear chain type, suggested use of DBS for slaughter house waste water treatment cannot create an additional pollution problem.

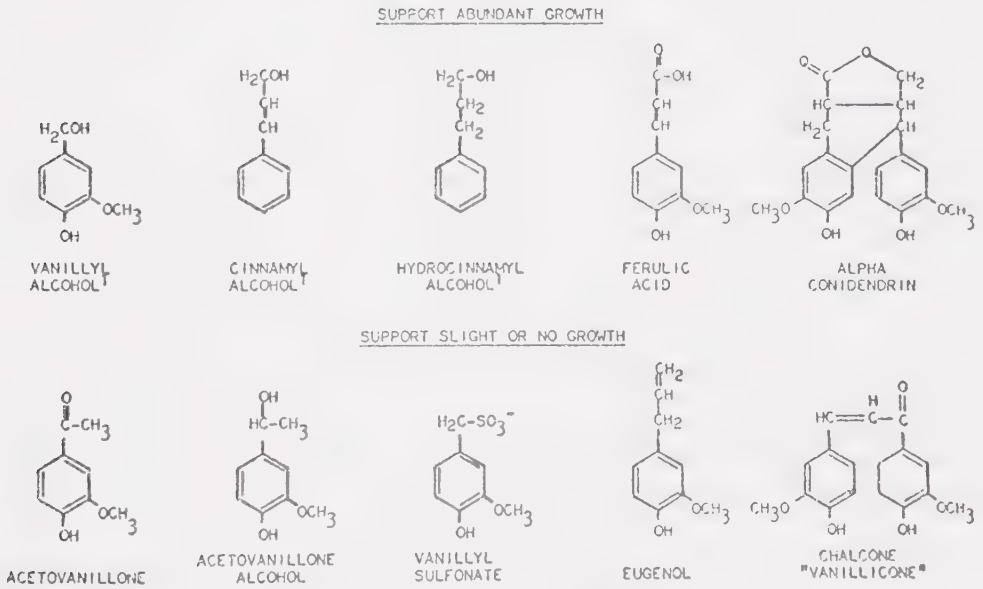


FIGURE VII.1 RELATIONSHIP BETWEEN STRUCTURE AND BIODEGRADABILITY OF LIGNIN COMPOUNDS

OBSERVATIONS AND RESULTS

The clarifications by the use of aluminum sulfate (alum) are presented in TABLES A.1, A.5 and FIGURES A.1, A.5. The plant "B" waste water from killing floor having a total solids content of 3,520 ppm after alum treatment was reduced to an average of 1,500 ppm. The corresponding ODI value after coagulation was slowly decreasing with the increasing quantity of alum used, reaching a value of 280 ppm with the highest doses of alum applied. The coagulation experiments were started at a pH value of 6.5, the pH of the untreated effluent. Similar tests were performed on combined effluent from the packing plants in which waste waters had a lower amount of total solids. From an average content of 2,600 ppm before coagulation the total solids after coagulation, with the highest doses of alum applied, were reduced to 1,780 ppm with a corresponding ODI reduction from 1,320 to 290 ppm.

The quantity of the alum applied for the combined effluent from the packing plants was approximately double of the amount used for plant "B" waste water. From both tests it appeared that the optimum amount of alum for the coagulation is approximately 300 ppm. This is especially obvious for plant "B" waste water and less so for the combined effluent from packing plants. In the latter case 300 ppm of alum results in an effluent with ODI value of 420 ppm. If the alum quantity is doubled the ODI quantity decreases only to 330 ppm which decrease does not justify the use of alum in a quantity above 300 ppm.

In these considerations the amount of total solids were omitted. If they were included in the considerations the optimun alum quantity of 300 ppm would be even more justified.

From the results obtained in this study and from previous results obtained by Bouthillier and Brown (6) it appears that about one third of total solids is present in a suspended form, and the remaining portion is dissolved. In other words, from a total solids content of 2,600 mg/l approximately 1,600 mg/l is present in the form of dissolved solids while the remaining 1,000 mg/l are suspended. By the methods used in this study about 90 % of suspended solids portion can be removed from waste water. Hence, from the aspect of suspended solids removal alum can be considered as a good coagulant.

The waste water coagulation using DBS as a flocculating agent is presented in TABLES A.2, A.6, and FIGURES A.2,A.6. The plant "B" waste water collected from killing floor was treated with a quantity of DBS ranging from 50 to 300 ppm. The combined effluents from packing plants having a lower total solids and ODI values were treated with a reduced quantity of DBS ranging from 25 to 175 ppm.

In both tests the coagulation has been done in an acidic media of pH 3.5. At the optimum amount of DBS applied, e.g. 100 ppm for plant "B" waste water and 50 ppm for combined effluents, the total solid content was reduced from 3,520 to 910 ppm for plant "B" waste and from 2,680 to 860 ppm for combined effluents.

Substantial reduction of ODI values has also been obtained.

The reduction for plant "B" waste water was from 1,800 to 100 ppm and for combined effluents from 1,320 to 70 ppm.

As presented in FIGURES A.9 and A.10 the ODI values in a supernatant liquor depend on the pH level at which the coagulation has taken place. The lowest pH value 2.0 was found to be less effective than the next values of pH 2.5 and 3.0. The optimum pH was found to be between 3.0 and 4.0. Further increases in pH caused an increase in ODI of the treated sample. For the pH of 4.5 the corresponding ODI was 400 ppm and for pH 6.0 the ODI increased to 2,200 ppm, almost a sixfold increase. A similar trend of total solids removal and dependance on the pH of the waste water has been obtained. The optimal decrease of total solids was obtained at pH 3.5. From this pH level, either lowering or increasing the pH resulted in the increase of total solids in the supernatant liquor.

The waste water coagulation with sodium lignin sulfonate (Orzan S) are presented in TABLES A.3, A.7 and FIGURES A.3, A.7. For the lowest ODI, using the plant "B" waste water, the required amount of Orzan S was 300 ppm while for the combined effluents it was 100 ppm. Applying the optimum quantity of Orzan S the total solids reduction amounted from 3,520 to 920 ppm for plant "B" waste water and from 2,680 to 890 ppm for combined effluents. The waste water coagulation with Orzan S was also done at pH 3.5.

Similar waste water treatment, using ammonium lignin sulfonate (Orzan A) instead of sodium salt, are presented in TABLES A.4, A.8 and

FIGURES A.4, A.8. The waste water containing higher total solids was treated by Orzan A in a quantity ranging from 200 ppm to 450 ppm while the combined effluents from packing plants, containing less total solids were treated with 50 to 300 ppm. For the packing plant "B" waste water the lowest ODI found was 290 ppm which corresponded to 300 ppm of Orzan A. Regarding the total solids content the best reduction was obtained with 100 ppm of Orzan A which quantity resulted in the slight increase of ODI from 140 ppm to 160 ppm. As for Orzan S, the coagulation has been done at pH 3.5.

As stated by Hopkins and Dutter (40) aluminum sulfate as a flocculant is only partially effective in a slaughter house waste water treatment. This statement was also confirmed by the results presented in TABLES A.9 and A.10. The lowest efficiency in ODI reduction and total solids removal has been obtained by using aluminum sulfate. The efficiency in ODI removal for plant "B" waste water was only 84 % while for the combined effluents it was only 68 %. Similarly, the total solids removal efficiencies were 58 % and 43 % , respectively.

An improved efficiency in ODI and total solids removals for the both waste water samples have been achieved by using DBS and lignin sulfonic salts. In comparison with alum, the improvement in ODI removal was 13 % to 14 % for plant "B" waste water, and 27 % for the combined effluent. The total solids removal was also improved by 16 % for plant "B" waste water and by 25 % for the combined effluent.

If the ODI values obtained by the flocculating agents themselves are not taken into consideration then the efficiency in ODI removal for DBS and Orzan salts are practically the same. That also applies for the efficiency of the total solids removal. Similarly, the two salts of lignin sulfonic acid do not differ significantly, suggesting that the cationic portion of the salt has no influence on the flocculation. Based on visual observations there were differences in the flocculating rate as well as in the size of flocks formed. The flocculation rate was the highest with DBS, followed by Orzan S and then by Orzan A. The particle size of the flocks were also greater by using DBS in comparison to the Orzan salts.

As presented in TABLE A.11 the total solids of combined effluents from packing plants have an average of 2,260 ppm from which amount lipids comprised 620 ppm, that is about 27 %. The gas liquid chromatography analyses of these lipids have revealed that the major fatty acids present are oleic, palmitic and stearic acids while the minor fatty acids present are lauric, myristic and lincleic, beside some other unidentified acids. Additional analysis for the iodine number of the lipid portion gave an IN = 33 which is very close to the iodine number range found for tallow-beef.

There is a significant difference in flocculating efficiency of alum, DBS and Orzan salts, related to the lipid portion of the total solids. (TABLE A.17). The amount of lipids found in the supernatant liquor after coagulation with alum was the highest (590ppm), which amount was approximately twice as high as that found for DBS (225 ppm).

On the other hand, Orzan S provided a supernatant liquor with a lipid content of 22 ppm. Finally, there was also a difference between Orzan A and Orzan S. The ammonium salt was more efficient in lipid removal than the sodium salt. How the ammonium salt increases the efficiency of fat removal is not clear. The Orzan A has a higher content of lignin sulfonic acid and it has a lower content of ash than Orzan S. (Appendix F). In addition, there is a significant difference in their hydrolysis patterns. While Orzan S solution produces the pH of 7.0 the Orzan A aqueous solution produces the pH of 4.0. In acidified media, as used in the coagulation experiments, the ammonium ion in the presence of sulfate ions, generated from the sulfuric acid used as acidulant, might act as ammonium sulfate, a well known coagulant.

A buffering capacity curve on FIGURE A.11 probably represents the reaction of the free fraction with the sulfuric acid added. As seen from the FIGURE All the curve representing the consumption of sulfuric acid consists of three sections. The first representing the consumption of sulfuric acid in the range of pH from 5.5 to 4.5 is such as expected for any base-acid neutralization curve. The second section in the range of pH 4.5 to 3.2 is an inflected one which reflects the interaction of the acid and protein present. The third section from pH 3.2 to 2.5 is again as expected for the behavior of an acid in aqueous solution.

CHAPTER IX

CONCLUSIONS

Analysis of the samples of packing house waste showed an average protein content of 22.1 %. Furthermore, it can be concluded that the protein and lipid content amounts to an average of 50 % of total solids which lie in the range of 3,000 mg/l. Such an amount is worth considering for recovery and use as a supplement in the manufacture of livestock feed.

The mineral fraction of solids in waste waters was also analyzed. The highest amount was that of sodium which far exceeded the amounts of magnesium, calcium, potassium, copper and iron. These results were expected due to extend use of sodium chloride in meat processing. Because of the high content of sodium chloride the waste water from packing plants cannot be recommended for irrigation use.

The best results in regard to coagulation of proteins and lipids were obtained under acidic conditions at pH 3.5 with either sodium lignin sulfonate or DBS used as a coagulation agent. The COI removal was in range of 97 % and total solids removal amounted to 74 %. There was no significant difference found in the coagulation ability of these two agents. However, preference could be given to sodium lignin sulfonate considering its use as a binder for animal feed pellets (24).

The economics of the above mentioned coagulation process were not treated in this thesis. Though there is a pilot plant in operation in the United

Kingdom and two full scale plants are under construction (36), no studies of the economics of the process has been published in available scientific and technical literature. Some authors (35), (36) evaluated this process against conventional biological treatment systems and concluded that the operating cost will average somewhere between 50 % and 75 % of those normally found. However, this view is not supported by sufficient data.

A thorough study of the economics of the industrial application of the protein and lipids recovery by this process is recommended for future research.

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APPENDIX A

DETAILED DATA AND PLOTS

TABLE A.1

TEST FOR OPTIMUM AMOUNT OF ALUM
(PLANT "B" WASTE WATER FROM KILLING FLOOR)

pH	Temp. °C	Quantity of Alum used (ppm)	Total Solids before coagulation (ppm)	Total Solids after coagulation (ppm)	ODI before coagul. (ppm)	ODI after coagul. (ppm)
6.5	20	100	3,520	1,840	1,800	760
6.5	20	150	3,520	1,650	1,800	510
6.5	20	200	3,520	1,500	1,800	480
6.5	20	250	3,520	1,490	1,800	300
6.5	20	300	3,520	1,470	1,800	280
6.5	20	350	3,520	1,500	1,800	280

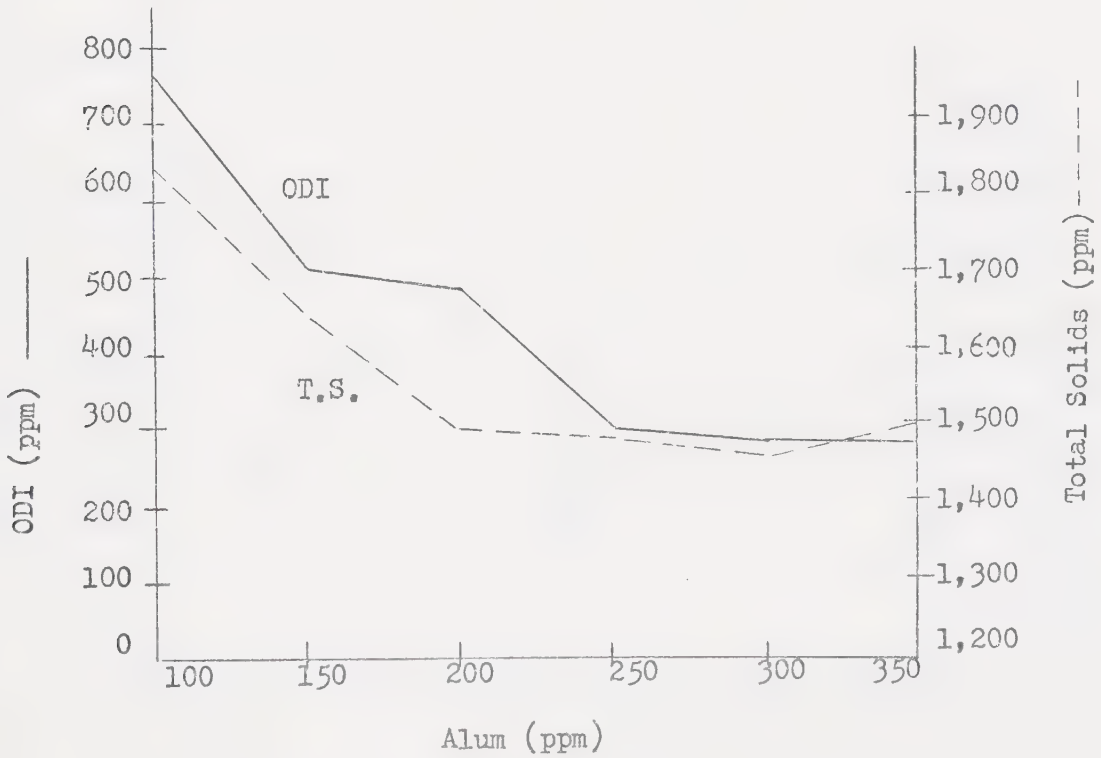


FIGURE A.1 TEST FOR OPTIMUM AMOUNT OF ALUM (PLANT "B" WASTE WATER FROM KILLING FLOOR)

TABLE A.2

TEST FOR OPTIMUM AMOUNT OF DBS
(PLANT "B" WASTE WATER FROM KILLING FLOOR)

pH	Temp. °C	Quantity of DBS used (ppm)	Total solids before coagulation (ppm)	Total Solids after coagulation (ppm)	ODI before coagul. (ppm)	ODI after coagul. (ppm)
3.5	20	50	3,520	1,200	1,800	260
3.5	20	100	3,520	910	1,800	100
3.5	20	150	3,520	910	1,800	100
3.5	20	200	3,520	920	1,800	140
3.5	20	250	3,520	1,050	1,800	160
3.5	20	300	3,520	1,130	1,800	190

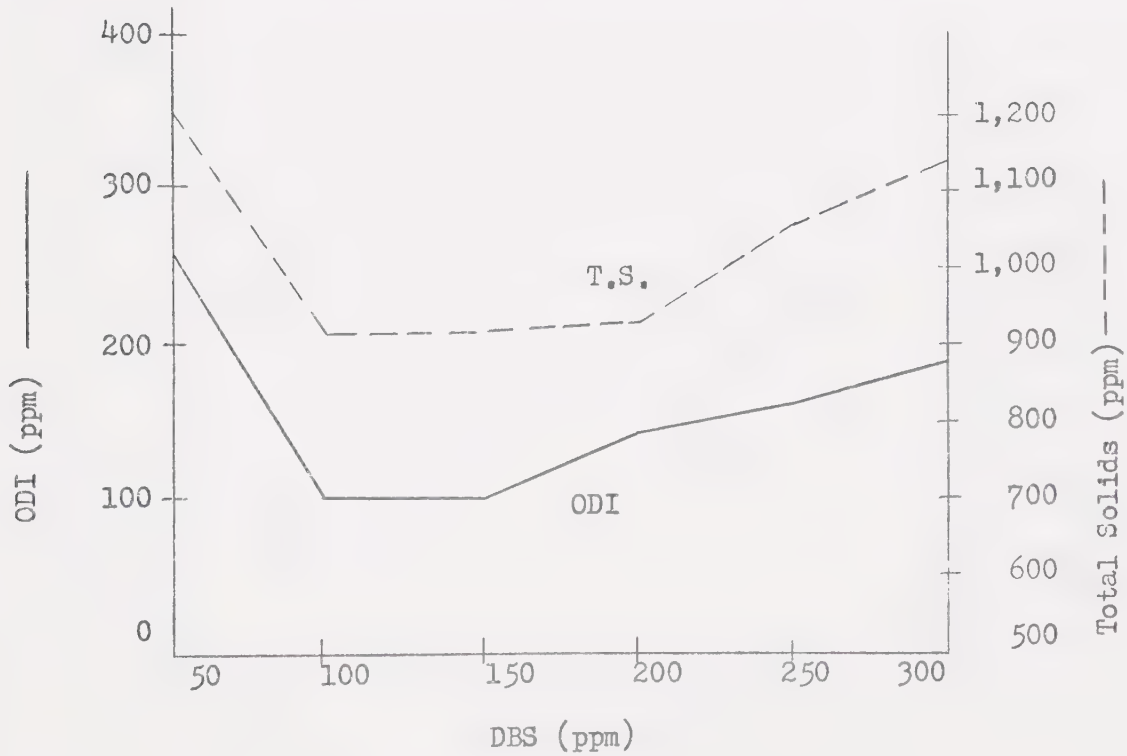


FIGURE A.2 TEST FOR OPTIMUM AMOUNT OF DBS (PLANT "B" WASTE WATER FROM KILLING FLOOR)

TABLE A.3

TEST FOR OPTIMUM AMOUNT OF ORZAN S
(PLANT "B" WASTE WATER FROM KILLING FLOOR)

pH	Temp. ^{o}C	Quantity of Orzan S used (ppm)	Total Solids before coagulation (ppm)	Total Solids after coagulation (ppm)	ODI before coagul. (ppm)	ODI after coagul. (ppm)
3.5	20	200	3,520	1,360	1,800	360
3.5	20	250	3,520	1,000	1,800	290
3.5	20	300	3,520	920	1,800	250
3.5	20	350	3,520	960	1,800	280
3.5	20	400	3,520	1,170	1,800	300
3.5	20	450	3,520	1,290	1,800	400

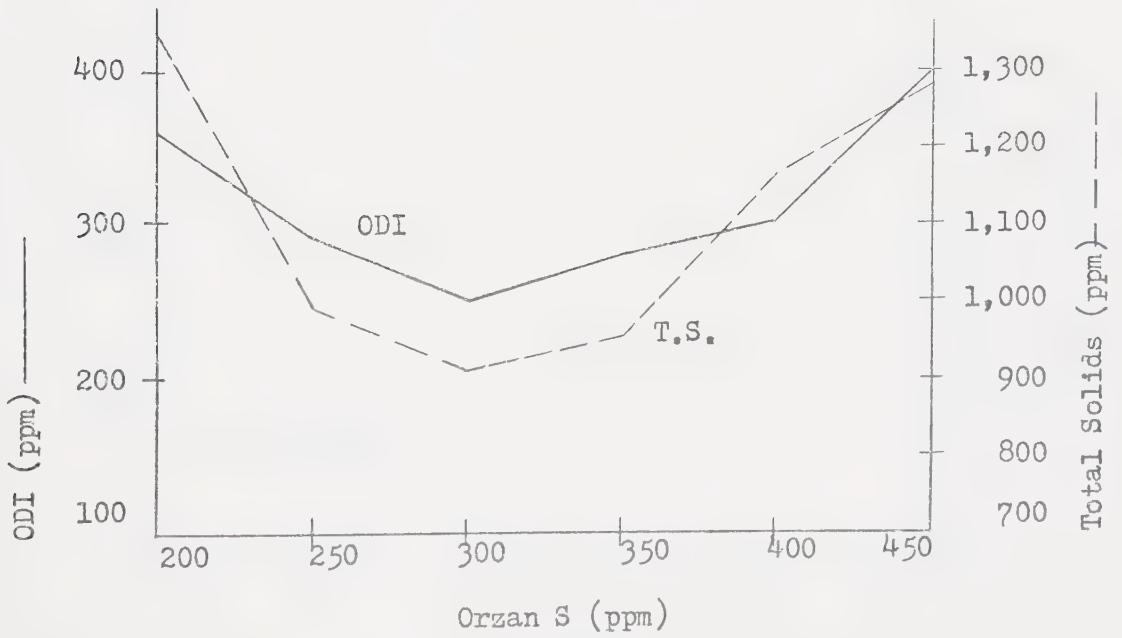


FIGURE A.3 TEST FOR OPTIMUM AMOUNT OF ORZAN S (PLANT "B" WASTE WATER FROM KILLING FLOOR)

TABLE A.4

TEST FOR OPTIMUM AMOUNT OF ORZAN A
(PLANT "B" WASTE WATER FROM KILLING FLOOR)

pH	Temp. °C	Quantity of Orzan A used (ppm)	Total Solids before coagulation (ppm)	Total Solids after coagulation (ppm)	ODI before coagul. (ppm)	ODI after coagul. (ppm)
3.5	20	200	3,520	1,410	1,800	400
3.5	20	250	3,520	1,100	1,800	380
3.5	20	300	3,520	1,050	1,800	290
3.5	20	350	3,520	1,160	1,800	300
3.5	20	400	3,520	1,300	1,800	330
3.5	20	450	3,520	1,420	1,800	360

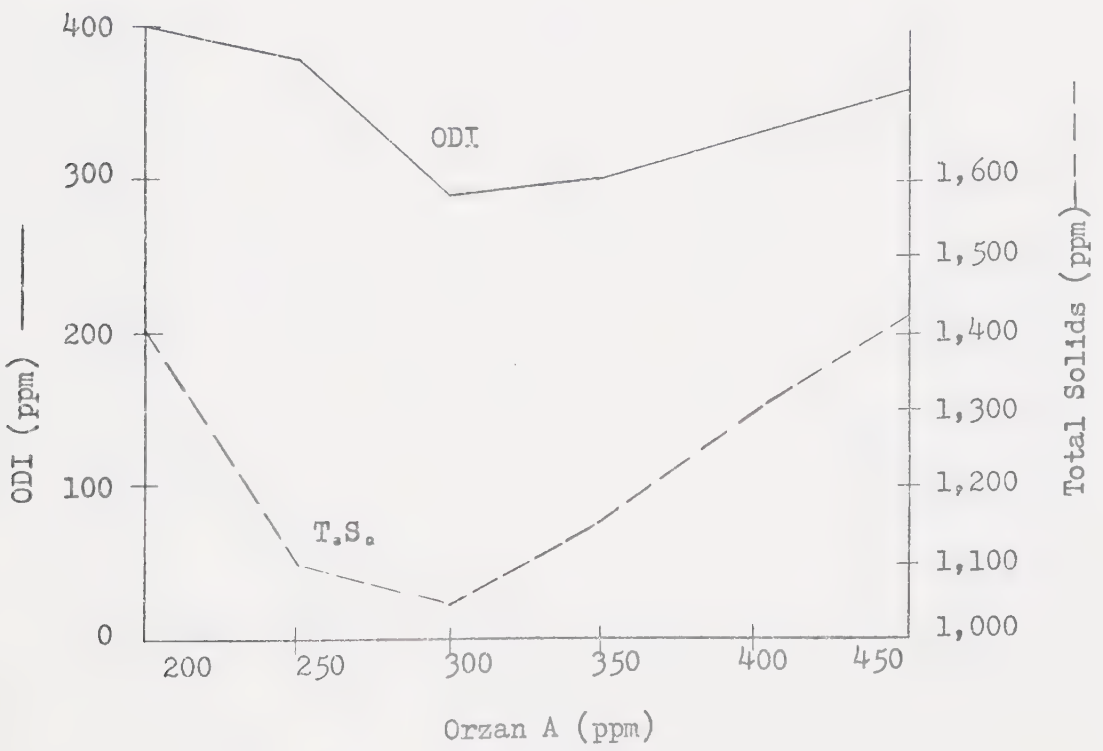


FIGURE A.4 TEST FOR OPTIMUM AMOUNT OF ORZAN A (PLANT "B" WASTE WATER FROM KILLING FLOOR)

TABLE A.5

TEST FOR OPTIMUM AMOUNT OF ALUM
(COMBINED EFFLUENTS FROM PACKING PLANTS)

pH	Temp. ^{o}C	Quantity of Alum used (ppm)	Total Solids before coagulation (ppm)	Total Solids after coagulation (ppm)	ODI before coagul. (ppm)	ODI after coagul. (ppm)
6.5	20	200	2,680	1,950	1,320	800
6.5	20	300	2,680	1,530	1,320	420
6.5	20	400	2,680	1,670	1,320	380
6.5	20	500	2,680	1,720	1,320	360
6.5	20	600	2,680	1,750	1,320	330
6.5	20	700	2,680	1,780	1,320	290

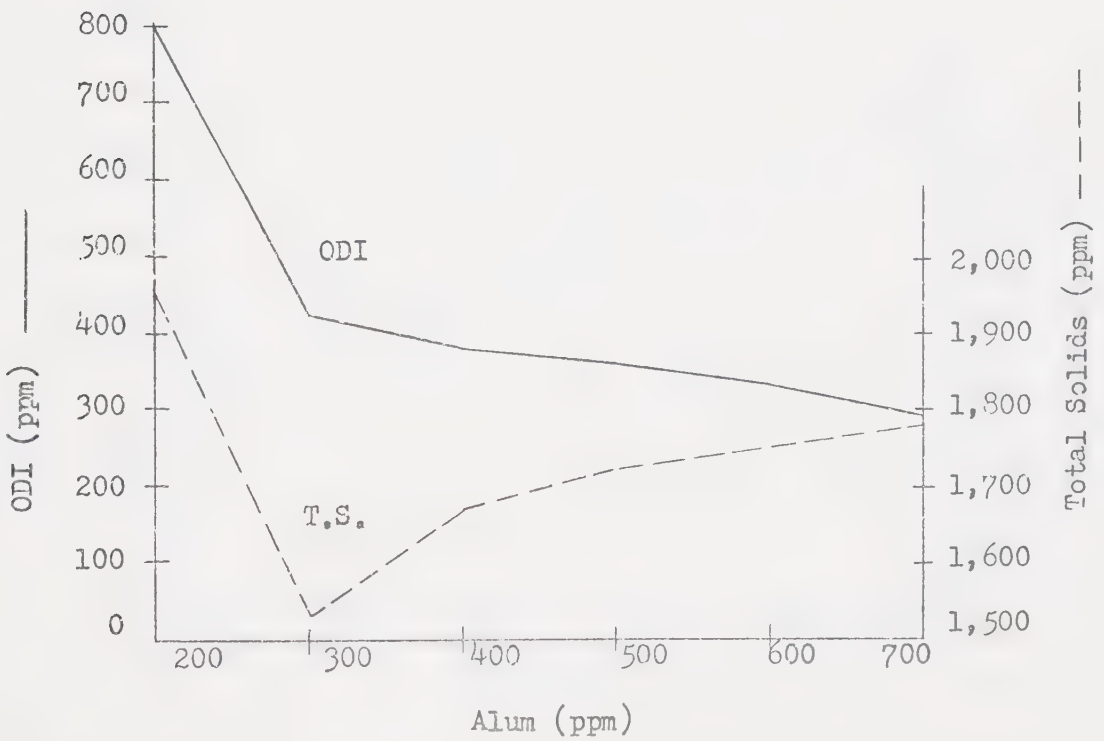


FIGURE A.5 TEST FOR OPTIMUM AMOUNT OF ALUM (COMBINED EFFLUENTS FROM PACKING PLANTS)

TABLE A.6

TEST FOR OPTIMUM AMOUNT OF DBS
(COMBINED EFFLUENTS FROM PACKING PLANTS)

pH	Temp. °C	Quantity of DBS used (ppm)	Total Solids before coagulation (ppm)	Total Solids after coagulation (ppm)	ODI before coagul. (ppm)	ODI after coagul. (ppm)
3.5	20	25	2,680	900	1,320	85
3.5	20	50	2,680	860	1,320	70
3.5	20	100	2,680	970	1,320	100
3.5	20	125	2,680	1,040	1,320	120
3.5	20	150	2,680	1,070	1,320	130
3.5	20	175	2,680	1,100	1,320	160

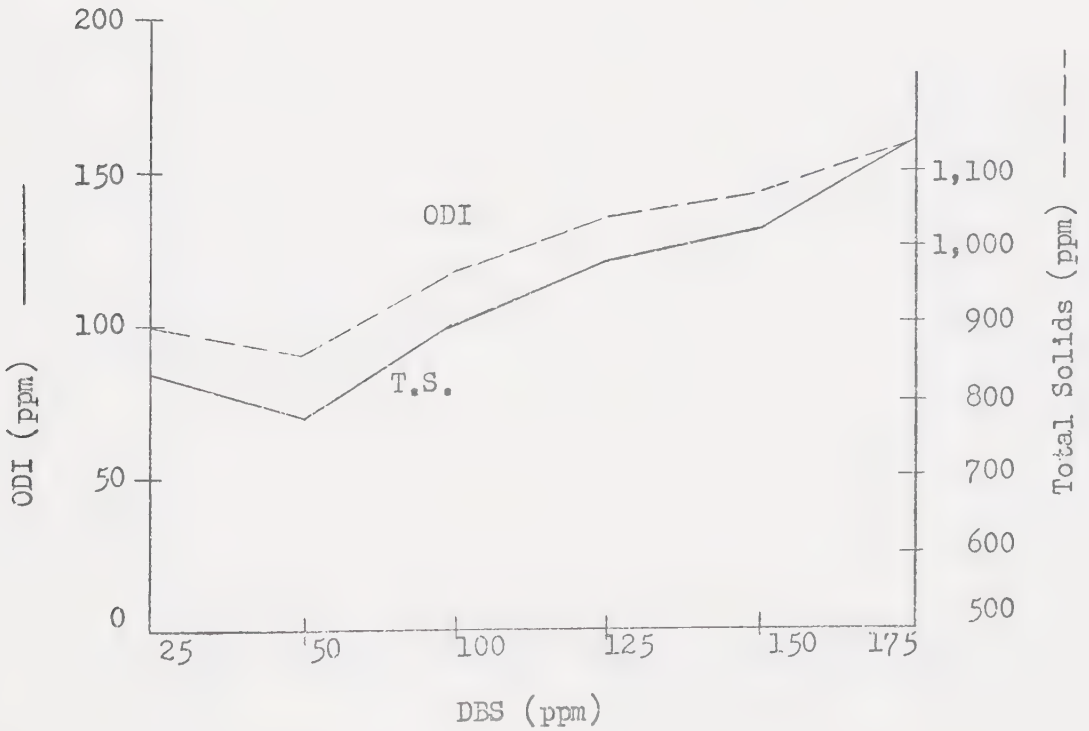


FIGURE A.6 TEST FOR OPTIMUM AMOUNT OF DBS (COMBINED EFFLUENTS FROM PACKING PLANTS)

TABLE A.7

TEST FOR OPTIMUM AMOUNT OF ORZAN S
(COMBINED EFFLUENTS FROM PACKING PLANTS)

pH	Temp. ^{o}C	Quantity of Orzan S used (ppm)	Total Solids before coagulation (ppm)	Total Solids after coagulation (ppm)	ODI before coagul. (ppm)	ODI after coagul. (ppm)
3.5	20	50	2,680	1,000	1,320	130
3.5	20	100	2,680	890	1,320	110
3.5	20	150	2,680	890	1,320	110
3.5	20	200	2,680	900	1,320	120
3.5	20	250	2,680	1,100	1,320	130
3.5	20	300	2,680	1,190	1,320	150

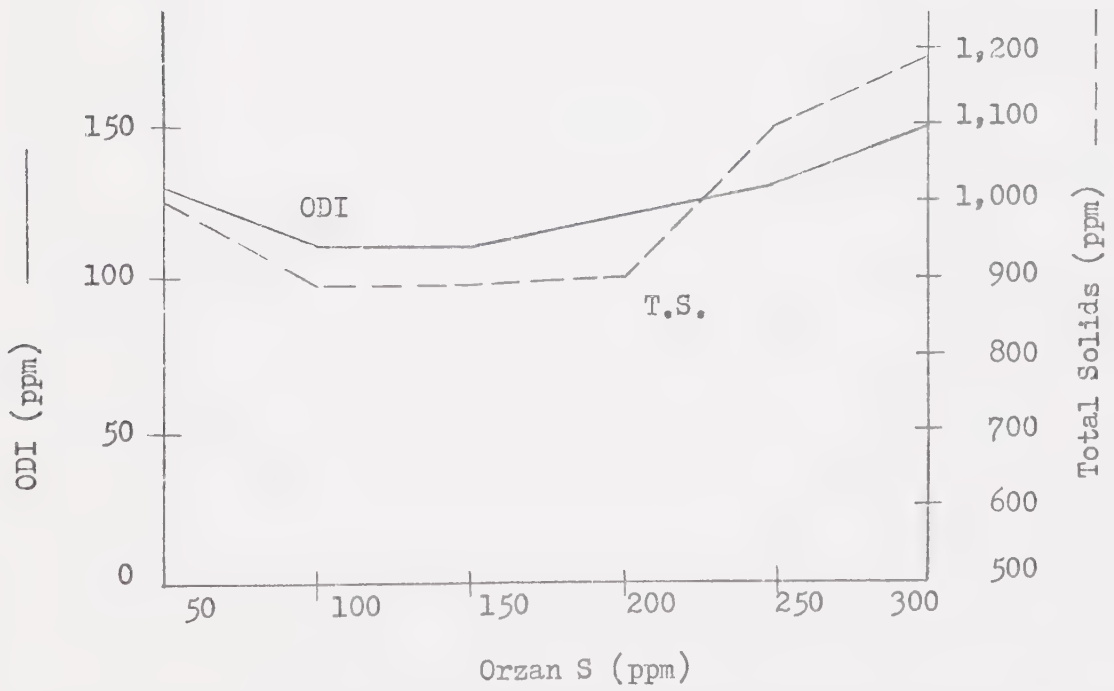


FIGURE A.7 TEST FOR OPTIMUM AMOUNT OF ORZAN S (COMBINED EFFLUENTS FROM PACKING PLANTS)

TABLE A.8

TEST FOR OPTIMUM AMOUNT OF ORZAN A
(COMBINED EFFLUENTS FROM PACKING PLANTS)

pH	Temp. °C	Quantity of Orzan A used (ppm)	Total Solids before coagulation (ppm)	Total Solids after coagulation (ppm)	ODI before coagul. (ppm)	ODI after coagul. (ppm)
3.5	20	50	2,680	1,280	1,320	140
3.5	20	100	2,680	1,070	1,320	160
3.5	20	150	2,680	1,100	1,320	170
3.5	20	200	2,680	1,220	1,320	190
3.5	20	250	2,680	1,320	1,320	200
3.5	20	300	2,680	1,410	1,320	240

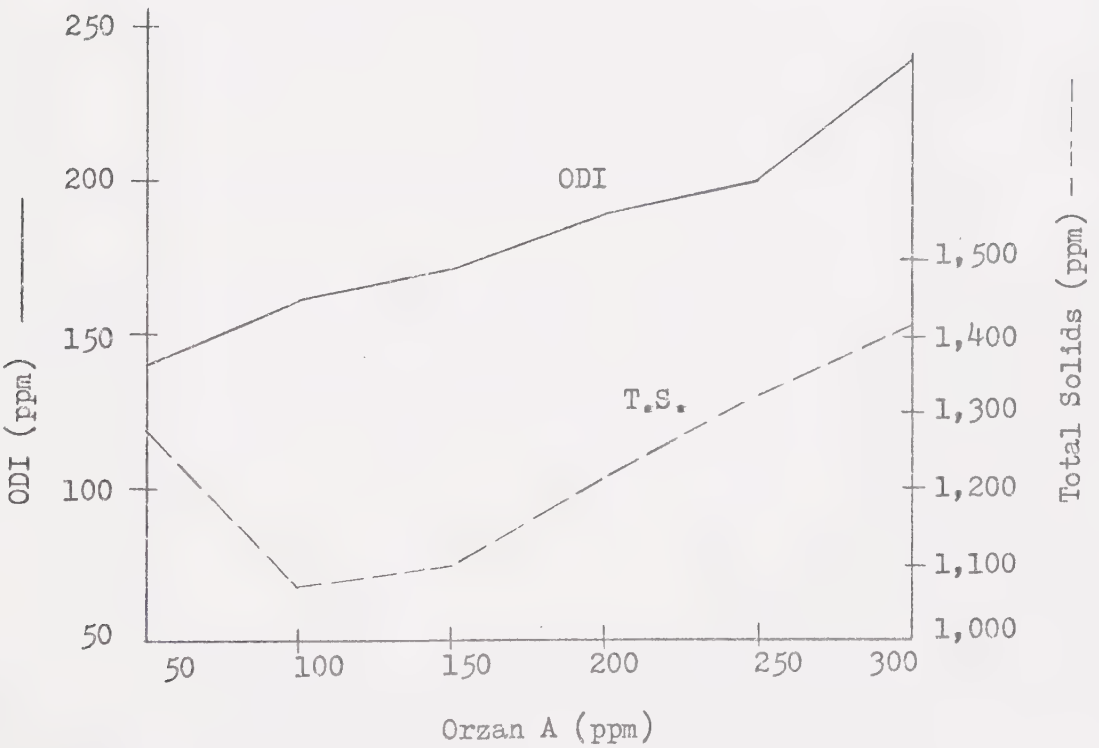


FIGURE A.8 TEST FOR OPTIMUM AMOUNT OF ORZAN A (COMBINED EFFLUENTS FROM PACKING PLANTS)

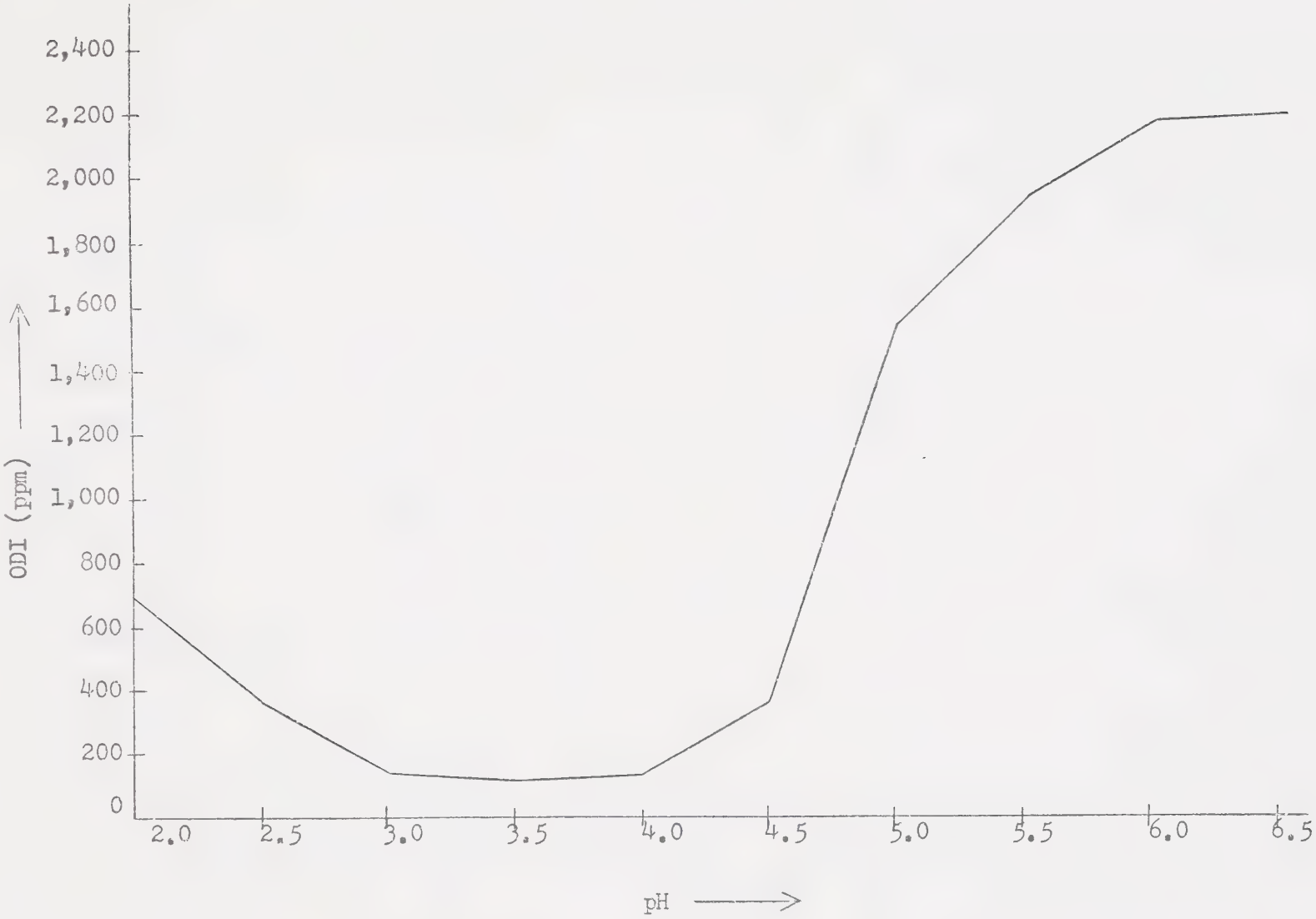


FIG. A.9 EFFECT OF PH LEVEL ON ODI IN SUPERNATANT LIQUOR (PLANT "B" WASTE WATER, 100 PPM OF DBS USED AS COAGULATING AGENT)

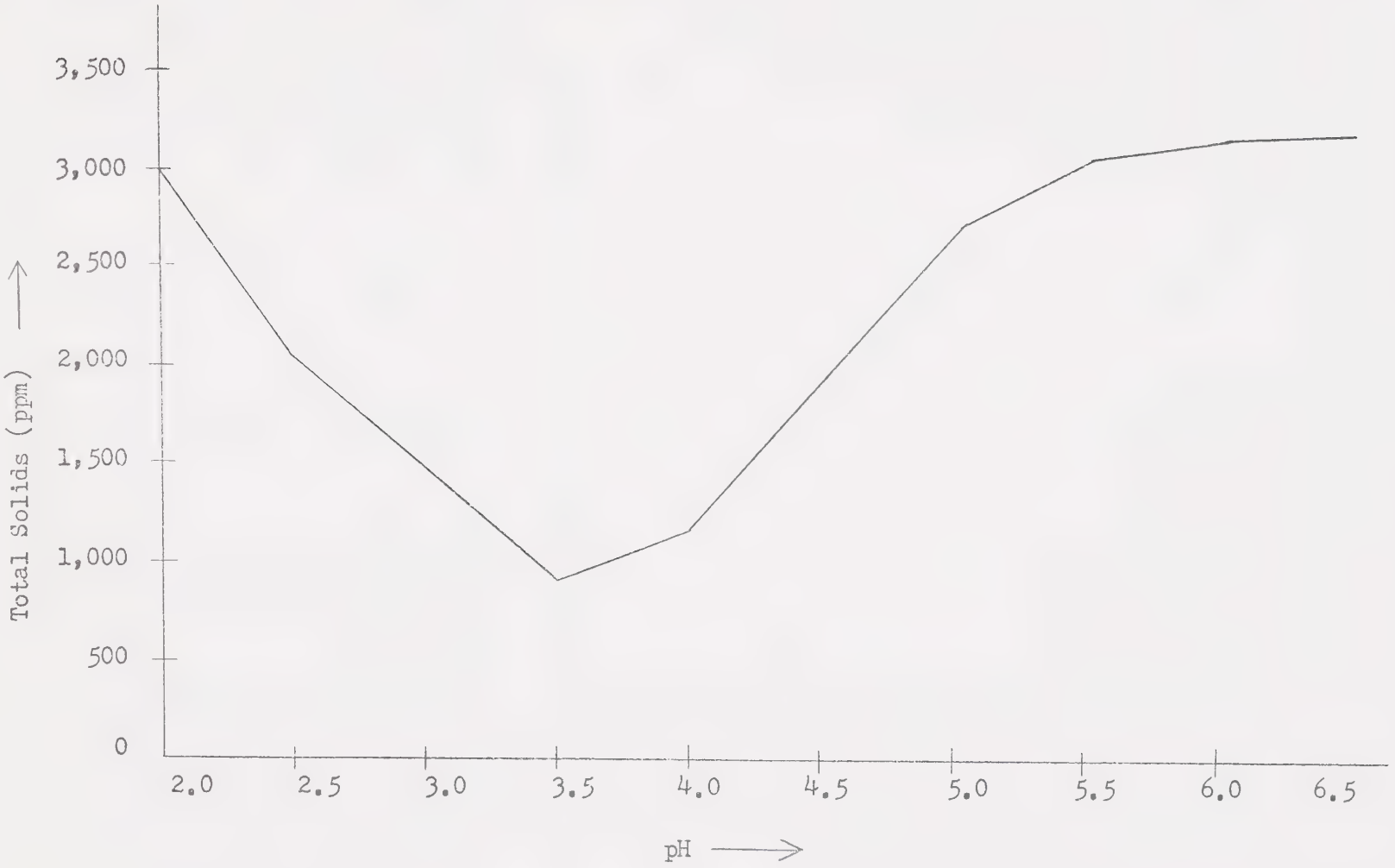


FIGURE A.10 EFFECT OF PH LEVEL ON AMOUNT OF TOTAL SOLIDS IN SUPERNATANT LIQUOR (50 PPM OF DBS USED AS COAGULATING AGENT)

TABLE A.9

EFFICIENCY OF ODI AND TOTAL SOLIDS REMOVAL
(PLANT "B" WASTE WATER FROM KILLING FLOOR)

Coagul. Agent	Quantity used (ppm)	ODI before coagul. (ppm)	ODI after coagul. (ppm)	T.S. before coagul. (ppm)	T.S. after coagul. (ppm)	ODI removal %	T.S. removal %
Alum	300	1,800	280	3,520	1,470	84	58
DBS	100	1,800	80	3,520	910	98	74
Orzan S	300	1,800	100	3,520	920	96	74
Orzan A	300	1,800	90	3,520	1,050	97	70

TABLE A.10

EFFICIENCY OF ODI AND TOTAL SOLIDS REMOVAL
(COMBINED EFFLUENTS FROM PACKING PLANTS)

Coagul. Agent	Quantity used (ppm)	ODI before coagul. (ppm)	ODI after coagul. (ppm)	T.S. before coagul. (ppm)	T.S. after coagul. (ppm)	ODI removal %	T.S. removal %
Alum	300	1,320	420	2,680	1,530	68	43
DBS	50	1,320	60	2,680	860	95	68
Orzan S	100	1,320	80	2,680	890	94	67
Orzan A	100	1,320	60	2,680	1,070	95	60

TABLE A.11

TEST DATA AND RESULTS
(24 HR COMPOSITE SAMPLES FROM EDMONTON PACKING PLANTS)

Month	Date	Packing Plant (ppm)	Total Solids (ppm)	Lipids (ppm)	ODI (ppm)	BOD (ppm)	TOC (ppm)
		A	2,530	344	560	957	521
August	8/71	B	3,660	382	750	1,116	608
		C	2,720	395	610	1,120	612
		A	1,545	282	780	1,630	567
Sept.	2/71	B	2,890	980	1,820	2,175	1,198
		C	1,975	380	1,120	1,250	907
		A	1,570	405	700	1,860	840
Sept.	28/71	B	1,730	437	950	1,312	590
		C	1,930	801	850	2,061	930
		A	2,160	1,278	1,125	1,730	680
Sept.	29/71	B	2,360	623	1,300	1,690	662
		C	2,900	1,288	1,300	2,172	983
		A	1,840	714	1,125	1,619	628
Sept.	30/71	B	1,900	642	1,225	1,845	832
		A	1,890	522	1,020	1,604	620
October	15/71	B	1,800	499	890	1,398	605
		C	3,200	1,687	1,570	2,472	1,050
		A	1,690	236	510	817	375
October	18/71	B	2,120	410	760	1,200	512
		C	2,600	426	760	1,203	538
		A	2,730	972	1,760	2,800	1,060
Nov.	17/71	B	1,500	290	1,080	1,701	766
		C	2,810	352	920	1,834	824
Average			2,260	620	1,030	1,630	735

ODI = 0.633 BOD, Standard Deviation = 0.123, Coefficient of Var. = 19.4 %

ODI = 1.401 TOC, Standard Deviation = 0.279, Coefficient of Var. = 19.9 %

Lipids = 27.4 % Total Solids

TABLE A.12

PROTEINS AND LIPIDS
(COMBINED EFFLUENTS FROM PACKING PLANTS)

Sample no.	Organic Nitrogen in T.S.	Proteins in T.S.	Organic Nitrogen in liquid effluent	Proteins in liquid effluent	Lipids in T.S.	Lipids in liquid effluent
1*	3.5%	21.8%	71 ppm	445 ppm	33.4%	680 ppm
2*	3.7%	23.1%	75 ppm	472 ppm	35.8%	730 ppm
3*	3.5%	21.8%	71 ppm	445 ppm	26.5%	540 ppm
4*	3.5%	21.8%	71 ppm	445 ppm	22.8%	465 ppm
Average	3.5%	22.1%	72 ppm	452 ppm	29.6%	604 ppm

* Grab samples taken 25/5, 7/7, 9/8 and 17/8 1971.

TABLE A.13

MINERAL COMPOSITION
(COMBINED EFFLUENTS FROM PACKING PLANTS)

Element	Sampling Date				Average
	May 5	Jul. 7	Aug, 9	Aug. 17	(ppm)
Na	314.0	226.0	283.0	460.0	321.0
Mg	7.0	4.2	5.5	3.9	5.1
Ca	15.1	18.0	20.0	13.2	16.5
K	18.8	9.0	14.0	19.0	15.2
Cu	Less than 0.5 ppm				0.0
Fe	1.9	1.2	0.9	1.0	1.2

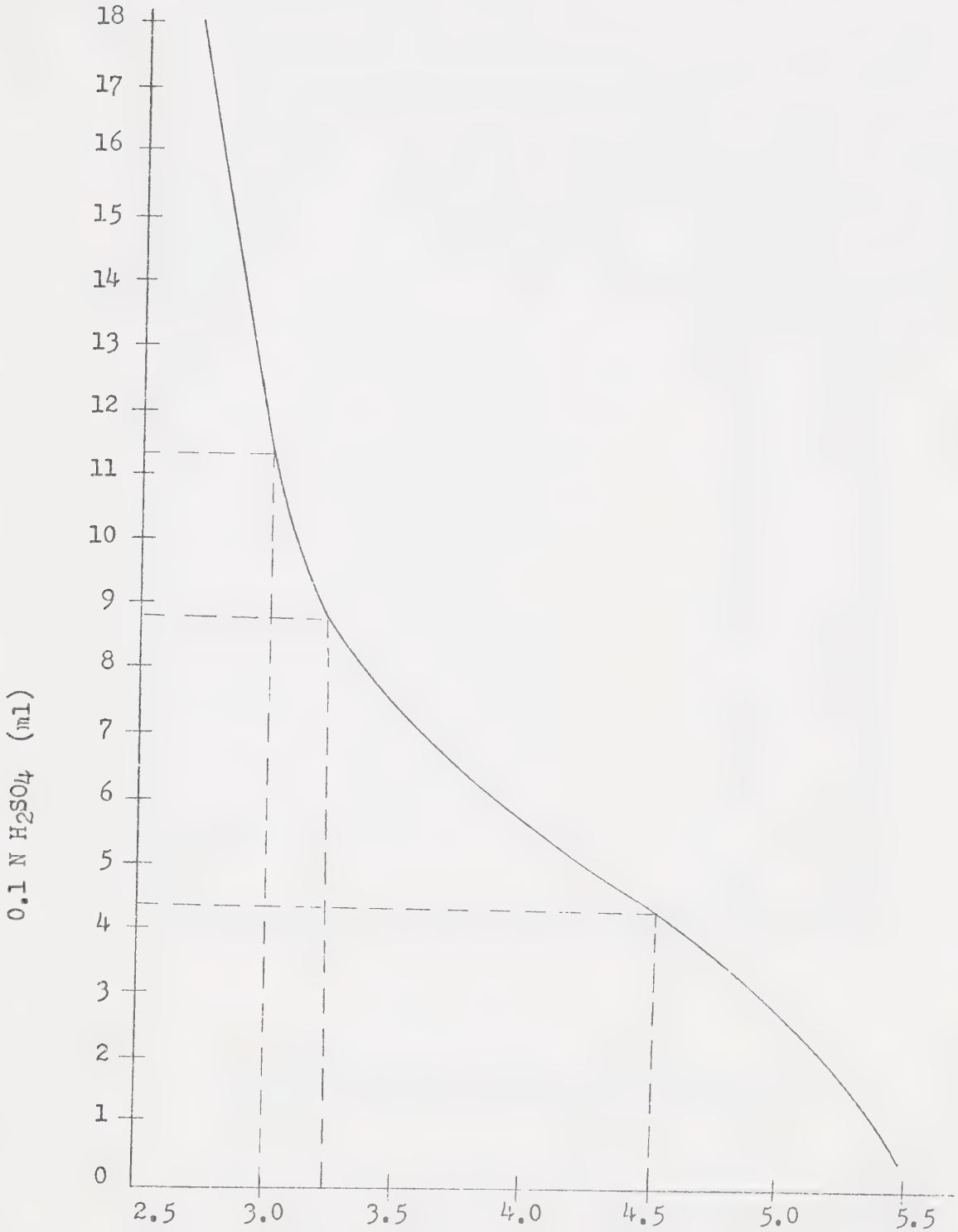


FIGURE A.11 CONSUMPTION OF SULFURIC ACID REQUIRED TO LOWER PH OF 100 ML OF COMBINED EFFLUENTS (INITIAL PH=6.5)

TABLE A. 14

DISTRIBUTION OF FATTY ACIDS
(COMBINED EFFLUENT FROM PACKING PLANTS)

Fatty Acid Designation	Calculation of Area from FIGURE A.12	Area (cm^2)	%
$C_{18:2}$	2.32x0.8x0.5	0.928	3.196
$C_{18:1}$	1.90x11.36x0.5	10.792	27.174
C_{18}	1.78x7.62x0.5	6.782	23.361
C_x	1.16x0.22x0.5	0.128	0.441
C_y	1.26x0.62x0.5	0.403	1.388
C_z	1.40x1.78x0.5	1.246	4.292
C_{16}	1.20x15.22x0.5	7.762	26.737
C_v	1.18x0.40x0.5	0.236	0.813
C_{14}	0.52x2.64x0.5	0.686	2.363
C_{12}	0.34x0.40x0.5	0.068	0.234
Total		29.031	99.999

TABLE A.15

COMPARISON OF SOME IODINE NUMBERS
(COMBINED EFFLUENTS FROM PACKING PLANTS)

Iodine no.	Lard	Tallow-beef	Tallow-mutton	Waste Sample
Iodine no. (typical)	73	40	40	33
Iodine no. (range)	65-80	35-50	35-46	33

TABLE A.16

COMPARISON OF DISTRIBUTION OF FATTY ACIDS

Acid name	Common Designation	% in Lard	% in Tallow-beef	% in Tallow-beef	% in Waste Sample *
Linoleic acid	C 18:2	14	2	5	3
Oleic acid	C 18:1	46	44	43	37
Stearic acid	C 18	9	16	30	23
Palmitic acid	C 16	23	35	21	27
Myristic acid	C 14	1	2	1	2
Lauric acid	C 12	0	0	0	0

* Percentage contained in Total Dry Solids of Combined Effluent from Packing Plants.

TABLE A.17

AMOUNT OF LIPIDS IN SUPERNATANT LIQUOR
(AFTER COAGULATION)

Coagulation Agent	Amount used	pH	Lipids
Alum	300 ppm	6.5	590 ppm
DBS	100 ppm	3.5	225 ppm
Orzan-S	300 ppm	3.5	25 ppm
Orzan-A	300 ppm	3.5	5 ppm

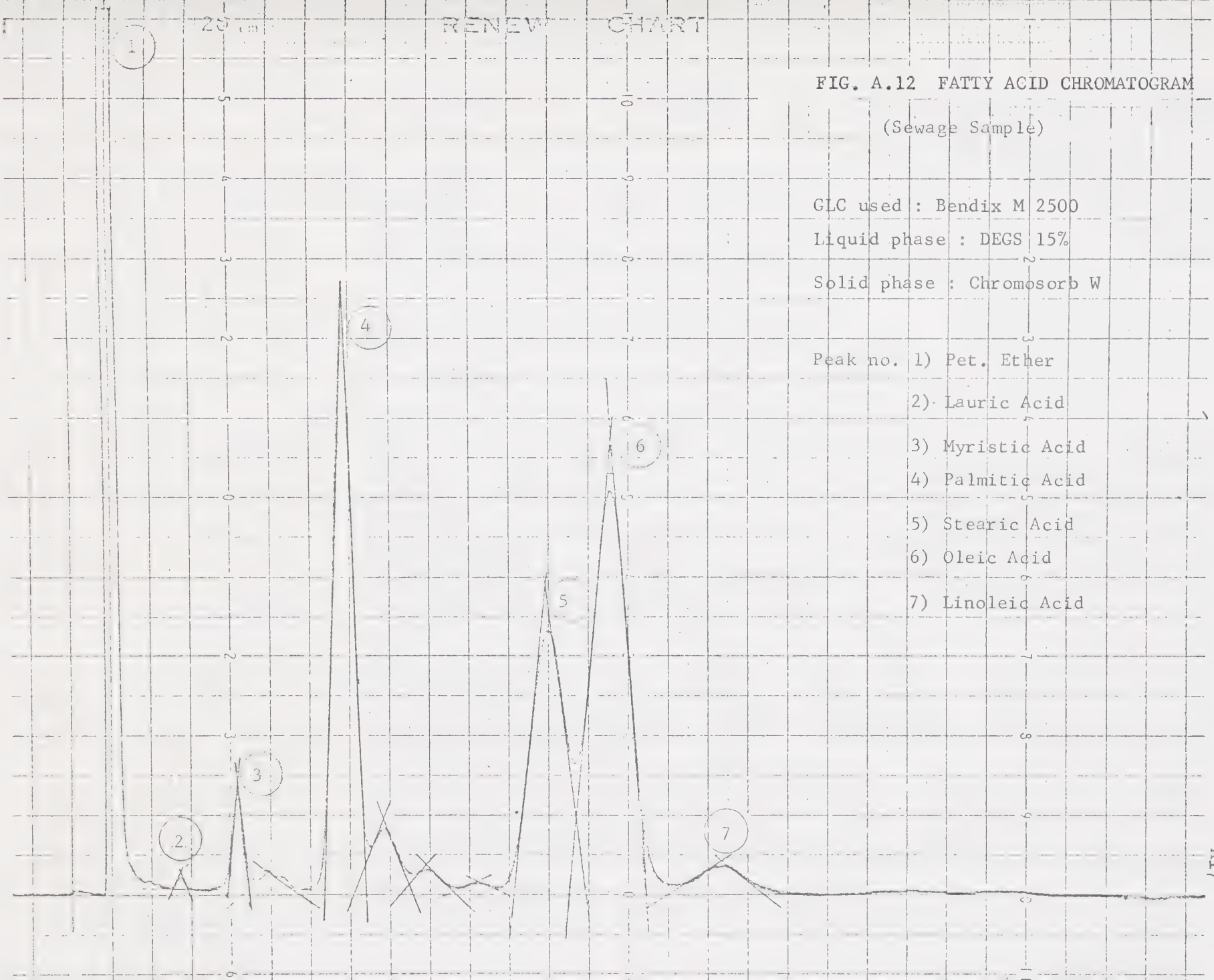


FIG. A.12 FATTY ACID CHROMATOGRAM

(Sewage Sample)

GLC used : Bendix M 2500

Liquid phase : DEGS 15%

Solid phase : Chromosorb W

Peak no. 1) Pet. Ether

2) Lauric Acid

3) Myristic Acid

4) Palmitic Acid

5) Stearic Acid

6) Oleic Acid

7) Linoleic Acid

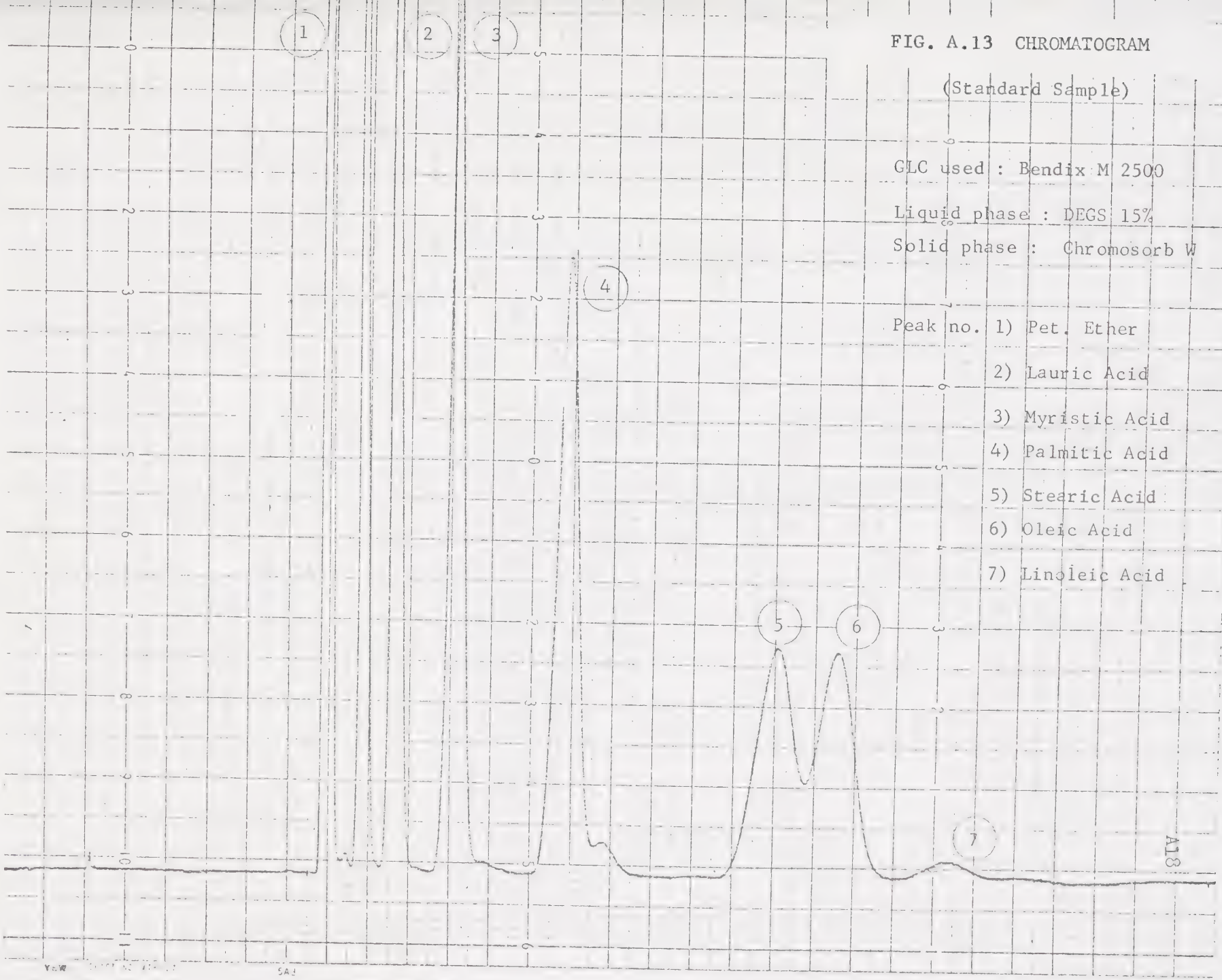


FIG. A.13 CHROMATOGRAM

(Standard Sample)

GLC used : Bendix M 2500

Liquid phase : DEGS 15%

Solid phase : Chromosorb W

Peak no. 1) Pet. Ether

2) Lauric Acid

3) Myristic Acid

4) Palmitic Acid

5) Stearic Acid

6) Oleic Acid

7) Linoleic Acid

APPENDIX B

FIGURES AND TABLES FROM LITERATURE CITED

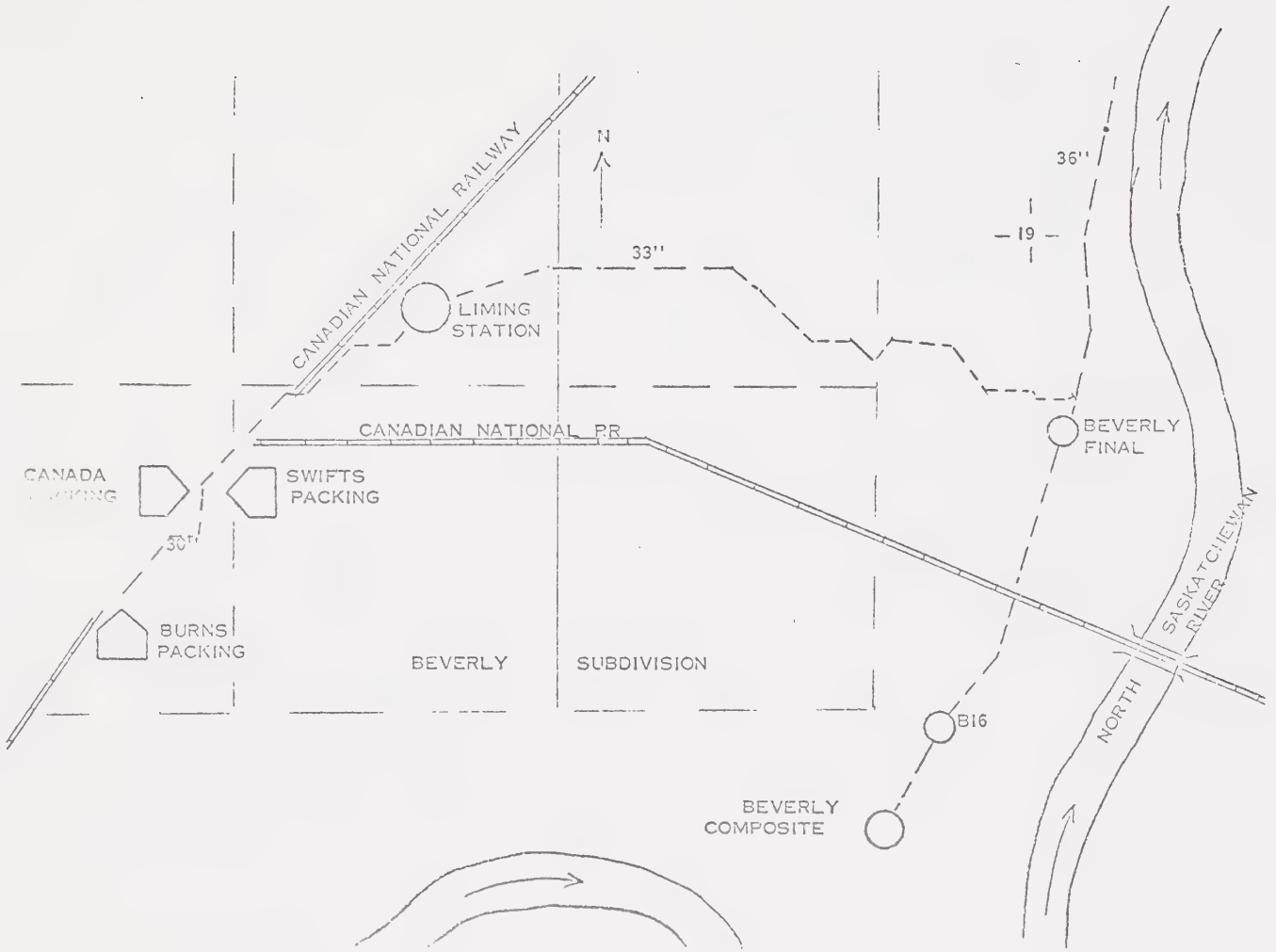


FIGURE B.1 PLOT PLAN SHOWING PACKING HOUSES IN EDMONTON

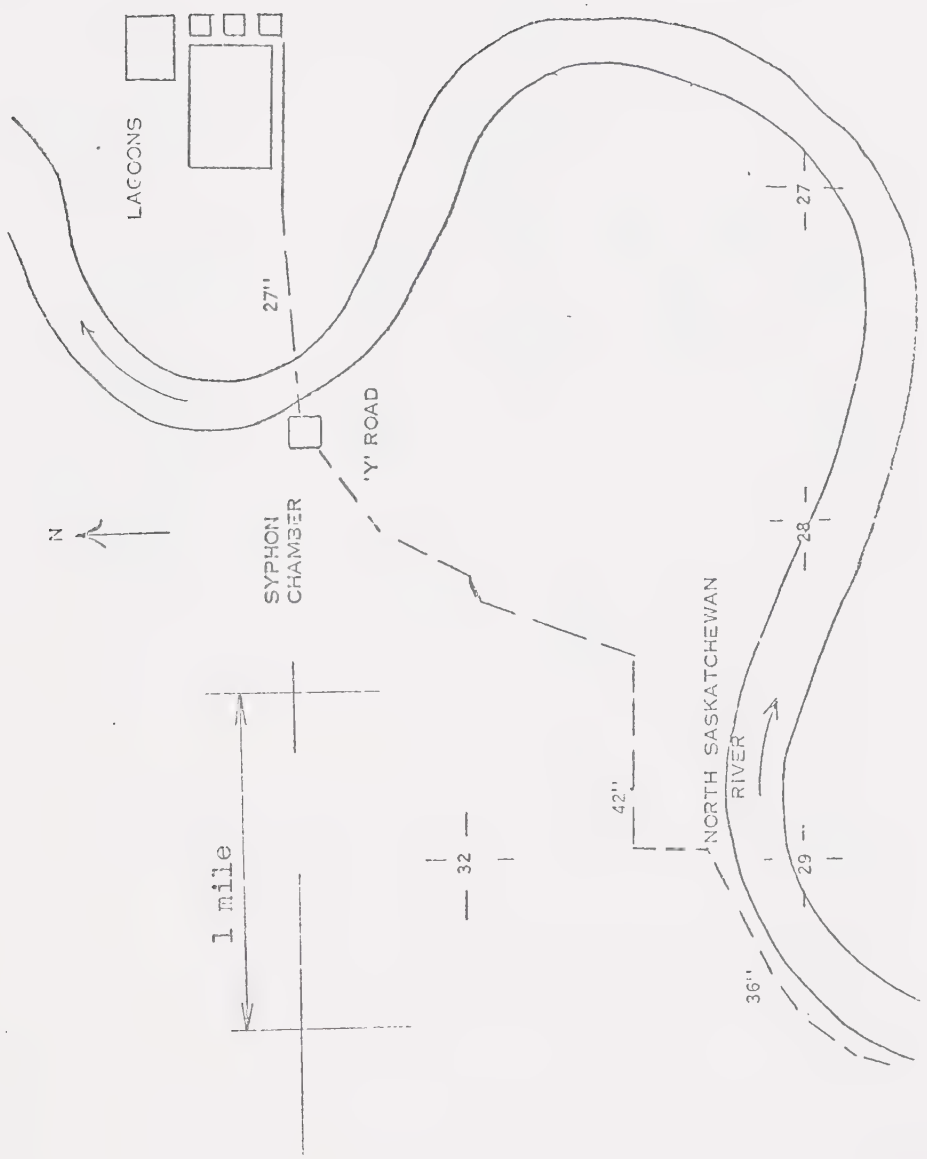


FIGURE B.2 LOWER PORTION OF COLLECTOR SEWER AND LAGOONS

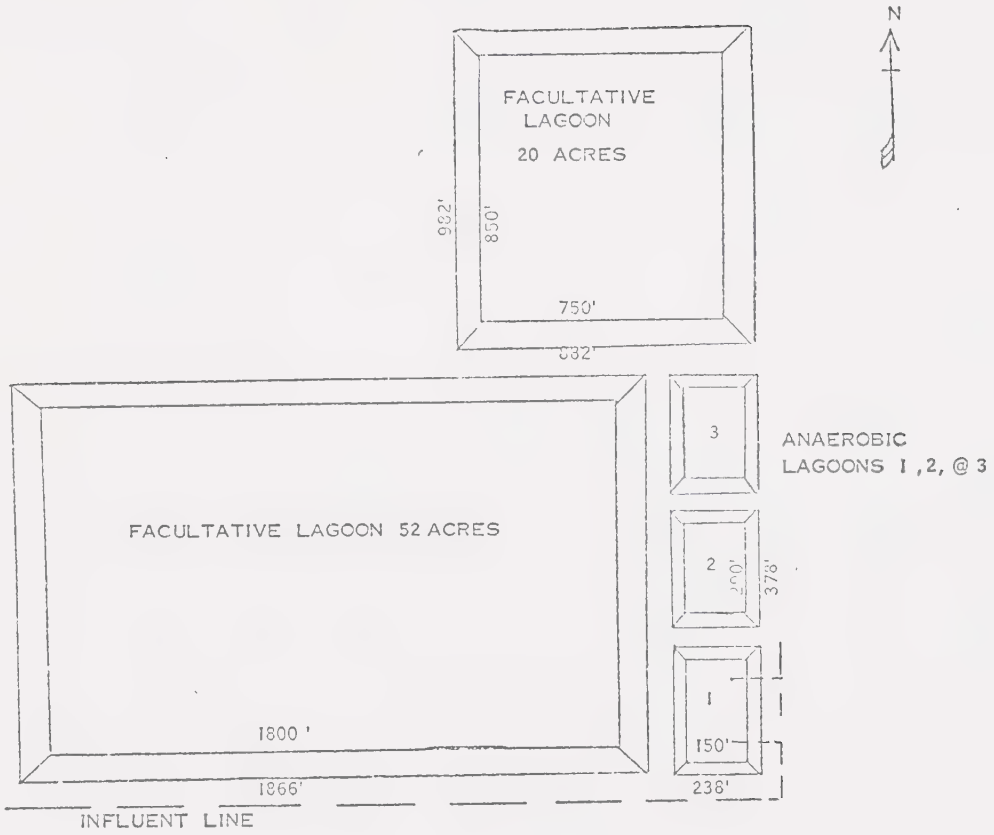


FIGURE B.3 PLOT PLAN OF EDMONTON WASTE LAGOONS

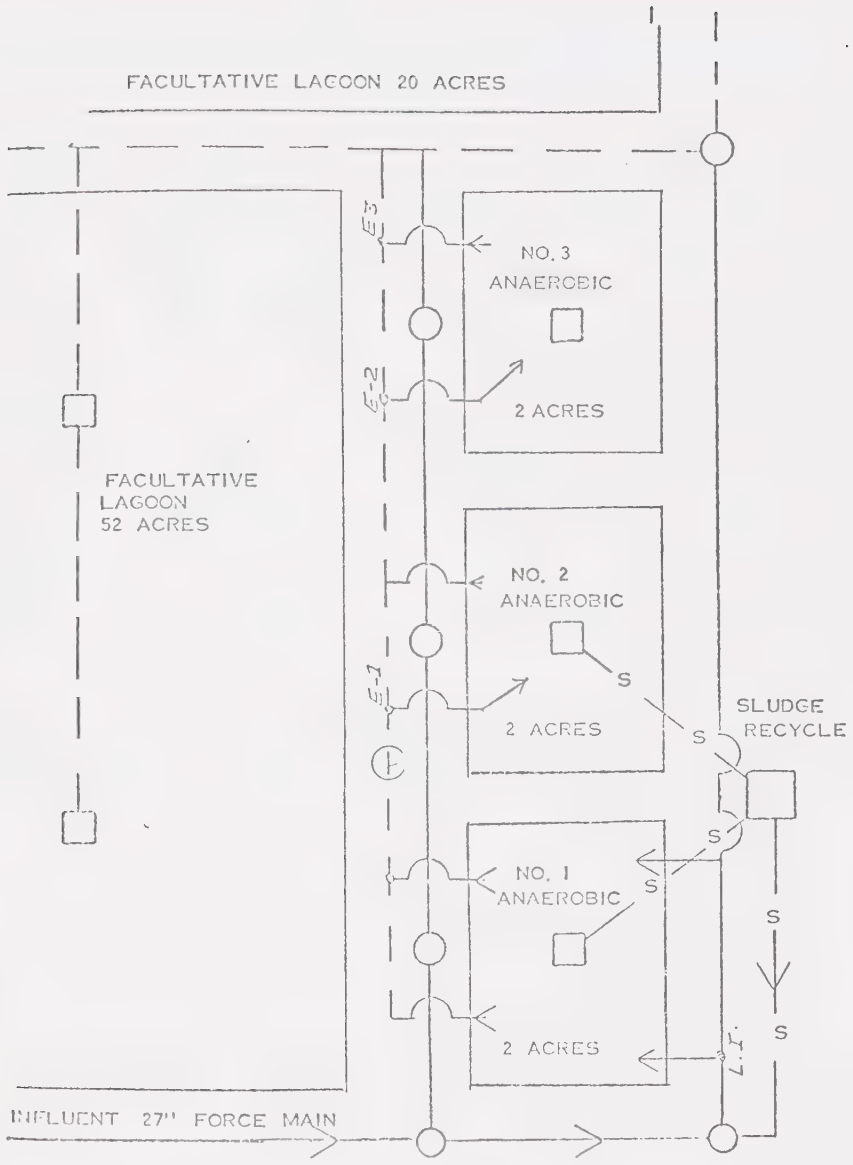


FIGURE B.4 SKETCH OF LAGOONS SHOWING INFLUENT AND PIPING AND SLUDGE RECIRCULATION LINES

TABLE B.1

APPROXIMATE RANGE OF FLOWS AND ANALYSES FOR SLAUGHTER HOUSES, PACKING HOUSES, AND PROCESSING PLANTS*

Operation	Waste flow Imp. gals per 1000 pounds live weight slaughtered	Typical analysis (mg/l) of waste water		
		BOD	Susp. Solids	Grease
Slaughter House	420-1700	2200-650	3000-930	1000-200
Packing House	600-3000	3000-400	2000-230	1000-200
Processing Plant	850-3500	800-200	800-200	300-100

* From "An Industrial Waste Guide to the Meat Industry," Public Health Service Publication No. 386, Revised 1965, p.6.

APPENDIX C

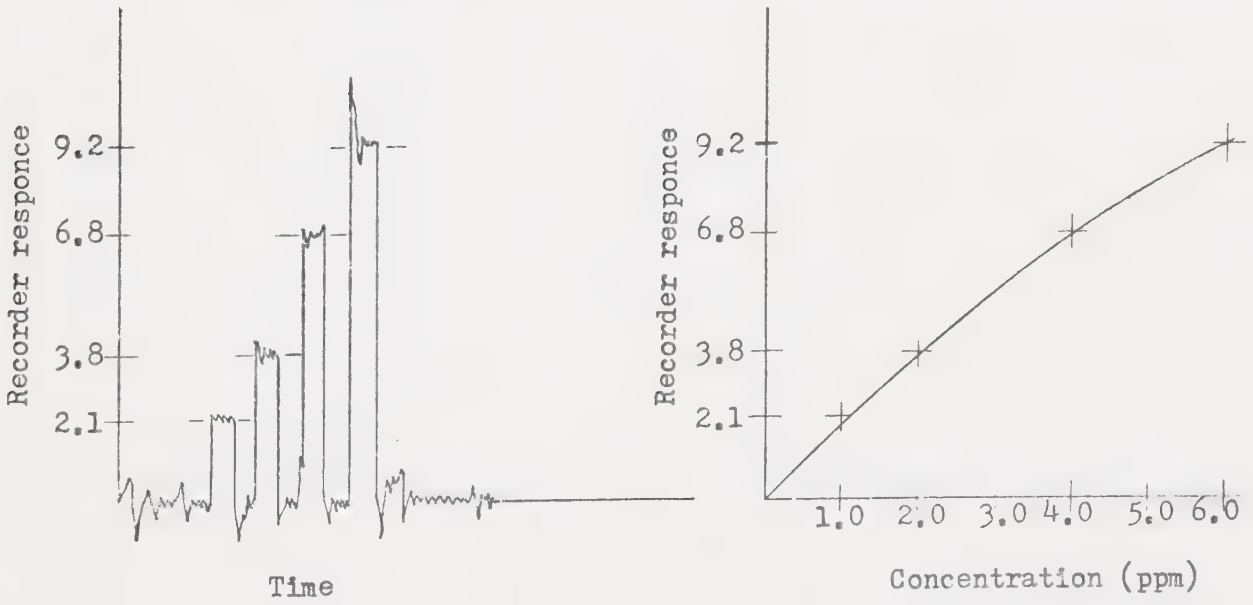
CALIBRATION CURVES FOR MACROELEMENTS AND MICROELEMENTS DETERMINATION

Calcium:

The hollow cathode lamp specification.

Fill gas	neon
Window	quartz
Operating current	4 mA
Strike voltage	260 V
Operating voltage	150 V
The line used	4226.7°A
Spectral band width	3.34 A
Sensitivity	0.03 ppm
Burner gas	oxygen-acetylene

Calibration Curves.

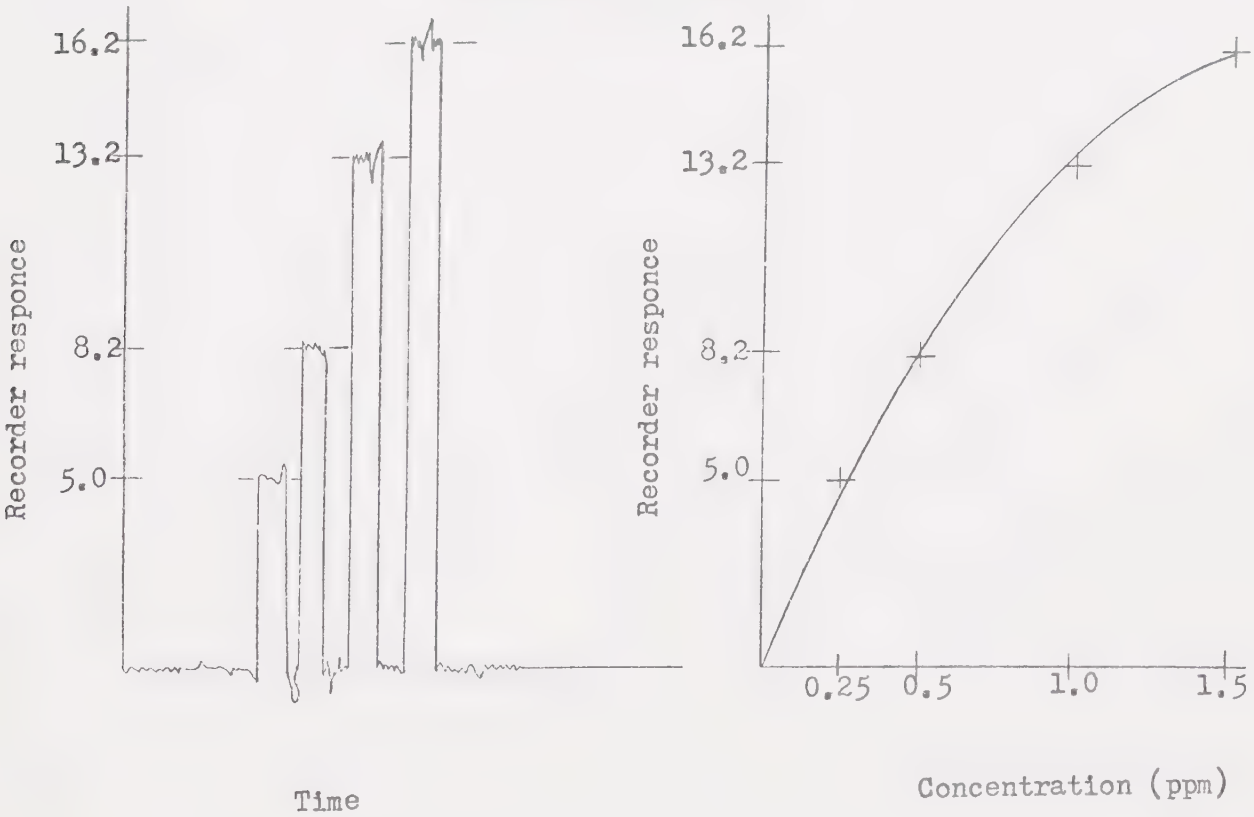


Magnesium:

The hollow cathode lamp specification.

Fill gas	neon
Window	quartz
Operating current	3 mA
Strike voltage	250 V
Operating voltage	150 V
The line used	2852.1°A
Spectral band width	3.3°A
Sensitivity	0.006 ppm
Burner gas	oxygen-acetylene

Calibration Curves.

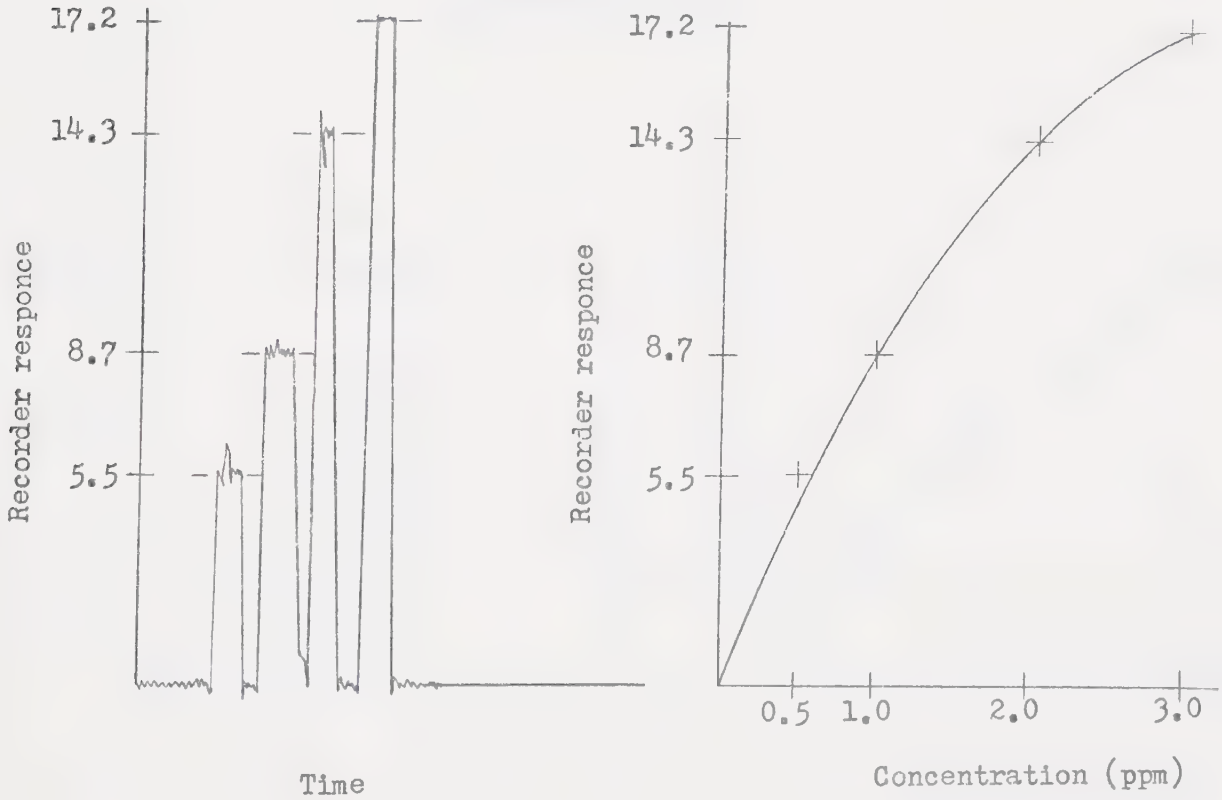


Sodium:

The hollow cathode lamp specification.

Fill gas	neon
Window	pyrex
Operating current	5 mA
Strike voltage	230 V
The line used	5890.0°A
Operating voltage	210 V
Spectral band width	6.6°A
Sensitivity	0.004 ppm
Burner gas	air-propane

Calibration Curves.

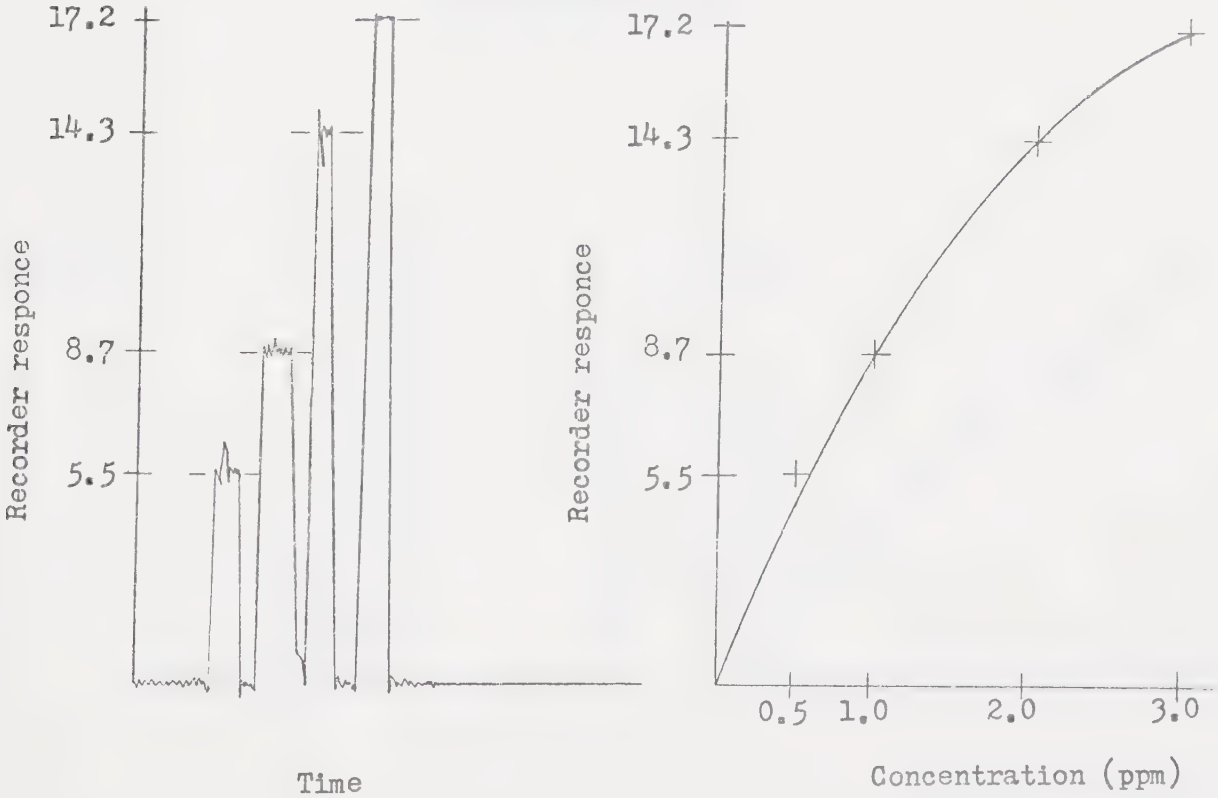


Sodium:

The hollow cathode lamp specification.

Fill gas	neon
Window	pyrex
Operating current	5 mA
Strike voltage	230 V
The line used	5890.0°A
Operating voltage	210 V
Spectral band width	6.6°A
Sensitivity	0.004 ppm
Burner gas	air-propane

Calibration Curves.

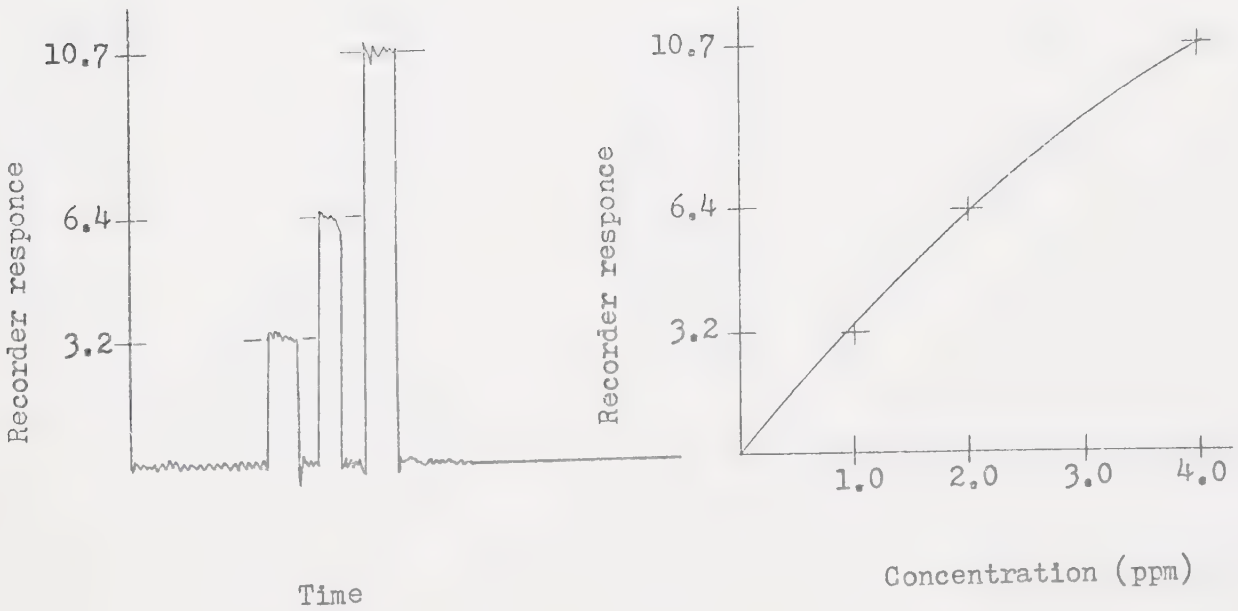


Potassium:

The hollow cathode lamp specification.

Fill gas	neon
Window	pyrex
Operating current	5 mA
Strike voltage	260 V
Operating voltage	180 V
The line used	7664.9°A
Spectral band width	6.6°A
Sensitivity	0.01 ppm
Burner gas	air-propane

Calibration Curves.

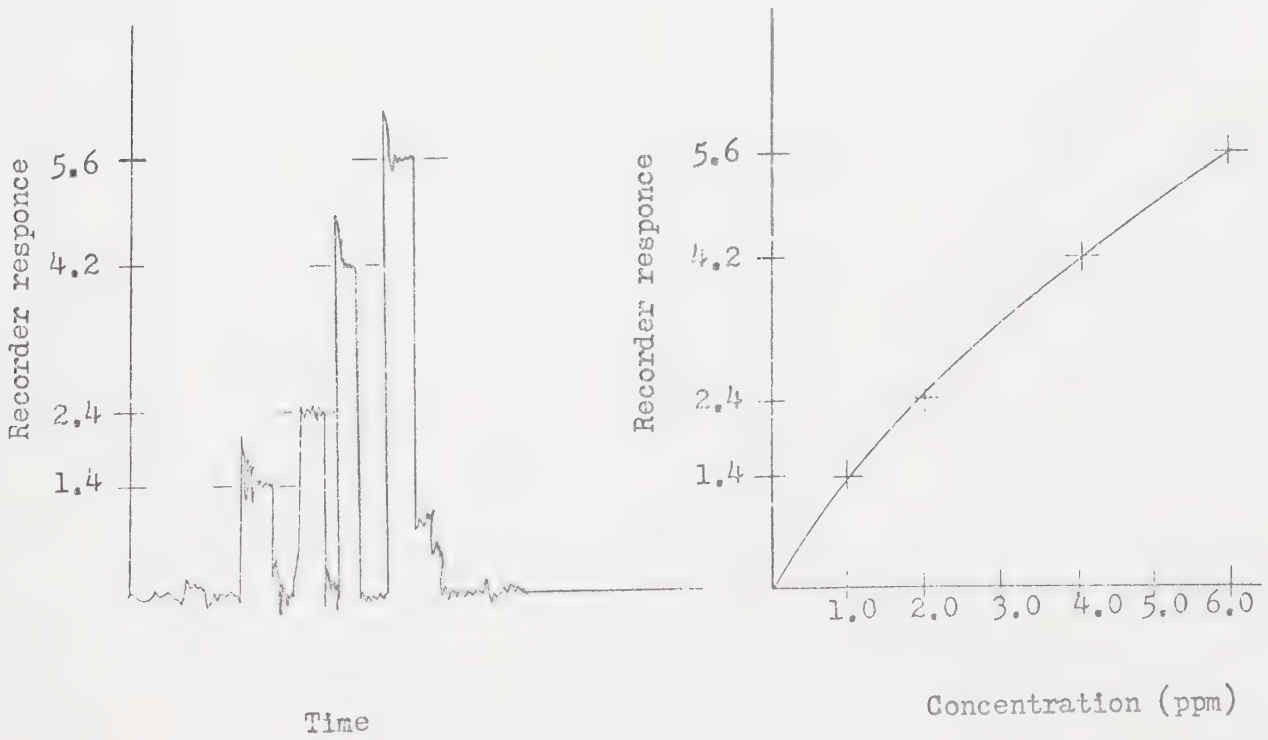


Iron:

The hollow cathode lamp specification.

Fill gas	neon
Window	quartz
Operating current	5 mA
Strike voltage	260 V
The line used	2483.3°A
Spectral band width	1.7°A
Sensitivity	0.03 ppm
Burner gas	oxygen-acetylene

Calibration Curves.

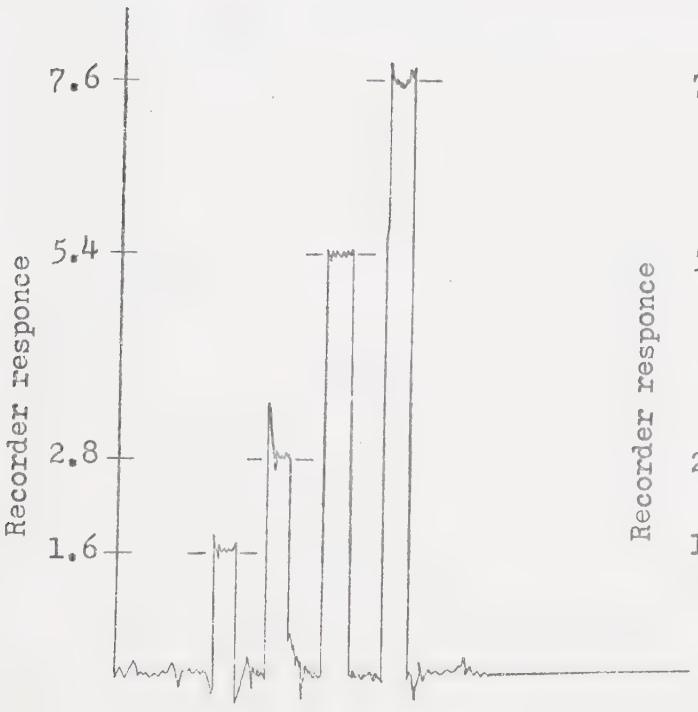


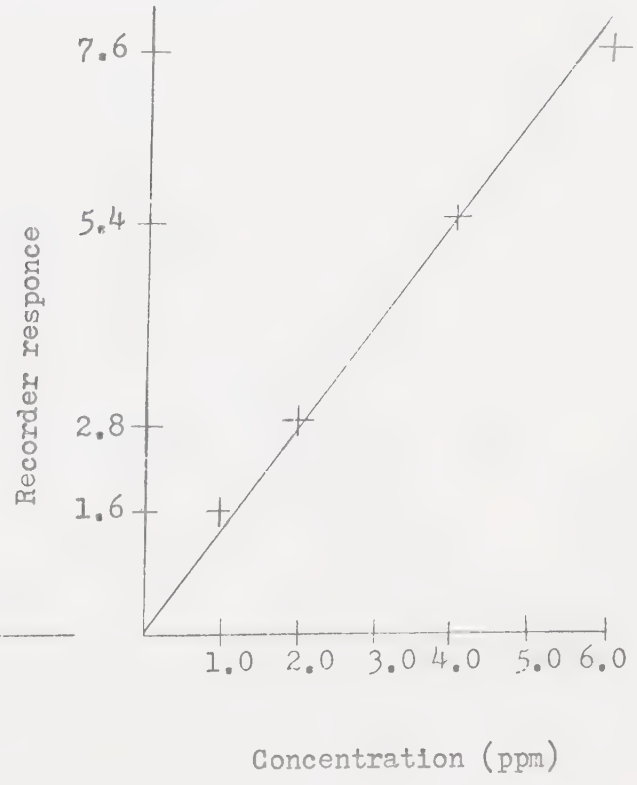
Copper:

The hollow cathode lamp specification.

Fill gas	neon
Window	quartz
Operating current	3 mA
Strike voltage	280 V
The line used	3247.5°A
Spectral band width	1.7°A
Sensitivity	0.04 ppm
Burner gas	oxygen-acetylene

Calibration Curves.





APPENDIX D

PROCEDURE FOR THE OXYGEN DEMAND INDEX

PROCEDURE FOR THE OXYGEN DEMAND INDEX

1. Reagents:

Potassium dichromate, a.r. ($K_2Cr_2O_7$)

Mercuric sulfate, a.r. ($HgSO_4$)

Glucose crystal, reagent or pure bacterial grade

Silver sulfate, a.r. (Ag_2SO_4)

Sulfuric acid, concentrated, a.r. (H_2SO_4)

Sulfuric acid - silver sulfate reagent: 22g of silver sulfate dissolved in 9 lb bottle of conc. sulfuric acid

2. Procedure:

Samples in a quantity of 5 ml were placed in 25x150 mm test tubes in a rack. (One test tube for blank was filled with 5 ml of distilled water). 0.1 g mercuric sulfate powder was added to each test tube and 1.5 ml of 0.25 N dichromate solution was pipetted to it. Then 7.5 ml of sulfuric acid - silver sulfate reagent was slowly pipetted into the tubes and the whole rack was placed into boiling water bath for 20 minutes. After cooling, the samples were read in a spectrophotometer "Spectronic 20" at 600 mu using blank for 100 percent. The ODI value was calculated from calibration curve.

3. Calibration Curve:

600 mg of glucose was dried for 1 hr at 103°C and then dissolved in 1,000 ml. This solution has theoretical BOD 450 mg/l. One ml of this solution has ODI value of 90 mg/l based on BOD test. Volumes of 1,2,3,4,5 ml glucose were pipetted into test tubes and distilled water was added up to the volume of 5 ml. Blank containing 5 ml of distilled water was prepared. 100 percent transmittance was set for blank at 600 mu. The graph was prepared by using samples containing 1,2,3,4,5 ml glucose and reading appropriate transmitance.
The calibration curve is shown in FIGURE D.1.

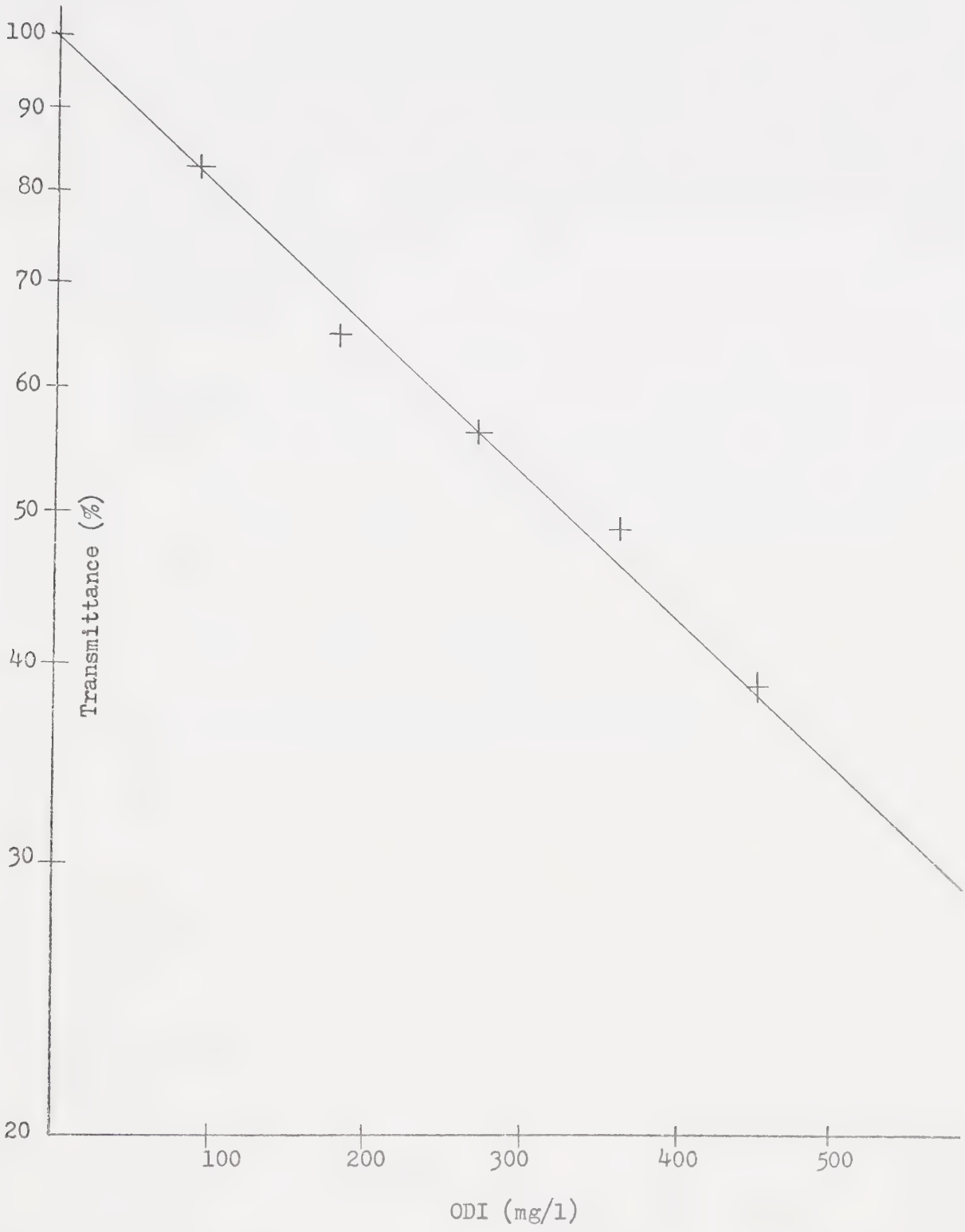


FIGURE D.1 CALIBRATION CURVE FOR ODI

APPENDIX E

DATA AND CALCULATION RESULTS FOR NUCLEAR-MAGNETIC RESONANCE TEST

NUCLEAR-MAGNETIC RESONANCE

1. Data Obtained From the Graph Shown in FIGURE E.1.

x = 5.3 mm y = 9.4 mm y + c = 10.2 mm z = 212.3 mm

c = 10.2 - 9.4 = 0.8 mm

2. Calculation and Results.

d = area per proton = 0.2528 y - 0.2542 x - 0.0014 c

$$v = \text{number of olefinic protons} = \frac{1.2653x - 0.2583y + 0.0014c}{d}$$

$$T = \text{total number of protons} = \frac{z}{d}$$

M = average molecular weight = 120.0 + 7.013 T + 6.006 v

$$IN = \text{iodine number} = \frac{12{,}691.0\ v}{M}$$

d = 0.2528 x 9.4 - 0.2542 x 5.3 - 0.0014 x 0.8 =

= 2.376 - 1.347 - 0.001 = 1.028

v = (6.706 - 2.428 + 0.001)/ 1.028 = 4.162

T = 212.3 / 1.028 = 206.52

M = 120.0 + 1448.3 + 24.9 = 1593.3

IN = (12691.0 x 4.162)/ 1593.3 = 33.15

FIGURE E.1 NUCLEAR MAGNETIC RESONANCE GRAPH

APPENDIX F

CHEMICALS USED FOR COAGULATION EXPERIMENTS

CHEMICALS USED FOR COAGULATION EXPERIMENTS

Sulfuric acid, concentrated, a.r. (H_2SO_4), s.g. = 1.83

Dodecyl benzene sulfonic acid (DBS):

Average of C atoms	13.8	
Sulfonic acid mixture	65 %	para compound
	9 %	ortho compound
	26 %	disulfonic acid
Free sulfuric acid	10 %	

Aluminum sulfate (Alum), a.r. ($Al_2(SO_4)_3 \times 18 H_2O$

Amonium lignin sulfonate (Orzan A):

Water content	6 %
Lignin sulfonic acids	57 %
Reducing sugars	15 %
Ash	1.5 %
Alkali-liberated ammonia	3 %
Primary cation	ammonia
pH of 25 % solution	4

Sodium lignin sulfonate (Orzan S):

Water content	5 %
Lignin sulfonic acids	48 %

Reducing sugars	12 %
Ash	20 %
Alkali-liberated ammonia	0.1 %
Primary cation	sodium
pH of 25 % solution	7
1 N sulfuric acid solution	28 ml of conc. H_2SO_4, s.g. = 1.83, dissolved in 1 liter of distilled water

APPENDIX G

MICRO-KJELDAHL METHOD

MICRO-KJELDAHL METOD

Reagents:

Sulphuric acid	Sp. gr. 1.84, N-free
Mercuric oxide	N-free
Potassium sulfate	N-free
$NaOH-Na_2S_2O_3$ solution	60 g NaOH and 5 g $Na_2S_2O_3.5H_2O$ dissolved in 100 ml of distilled water
Boric acid solution	Standard solution (saturated)
Indicator solution	Methyl red-methylene blue
Hydrochloric acid	0.02 normal

Procedure:

The sample was transfered to 30 ml digestion flask and the following chemicals were added: 1.9 mg K_2SO_4, 40 mg HgO and 2ml of H_2SO_4. For each 10 mg of dry organic matter greater than 15 mg, an additional amount of 0.1 ml H_2SO_4 was added. The sample was heated to boiling and digested on a digestion rack for 1.5 hr. To a cool digested sample a minimum quantity of H_2O necessary to dissolve solids was added. Then the sample was transfered to a distillation aparatus containing a $NaOH-Na_2S_2O_3$ solution, in a quantity of 8-10 ml. An erlenmeyer flask containing 5 ml of saturated H_3BO_3 solution and 2-4 drops of indicator solution was placed under a condensor with the tip extending below the surface of solution.

After collection of about 15 ml of distillate and dillution to about 50 ml, the distillate was titrated with 0.02 N hydrochloric acid to the end point of methyl red-methylene blue. The calculation of nitrogen and protein amounts were done using the following formulas:

$$\% \text{ N} = \frac{(\text{ml HCl}) \times (\text{normality} \times 14.00 \times 100)}{\text{mg of sample}}$$

$$\% \text{ Protein} = (\%\text{N}) \times 6.25$$